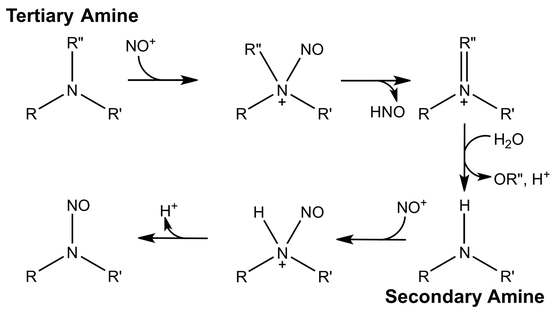
Constructing a Database of Nitrosation Reactions to Confirm and Expand (Q)SAR Model Predictions

K. Reiss, S. Chakravarti, and R. Saiakhov
MultiCASE Inc., Mayfield Heights, OH

GTA 2025

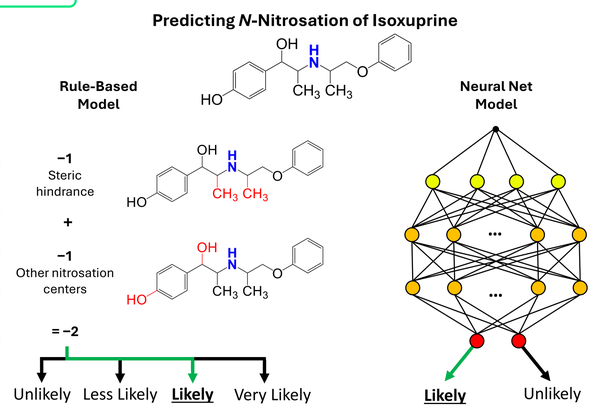
Background & Purpose

Since NDMA was discovered in pharmaceuticals in 2018, there has been a need for tools that can efficiently predict which compounds are will yield nitrosamines. Our goal was to build a (Q)SAR model to predict if (a) an amine is likely to be nitrosated and (b) what nitrosamines would be produced. We also sought to create a searchable database of existing nitrosation reaction.

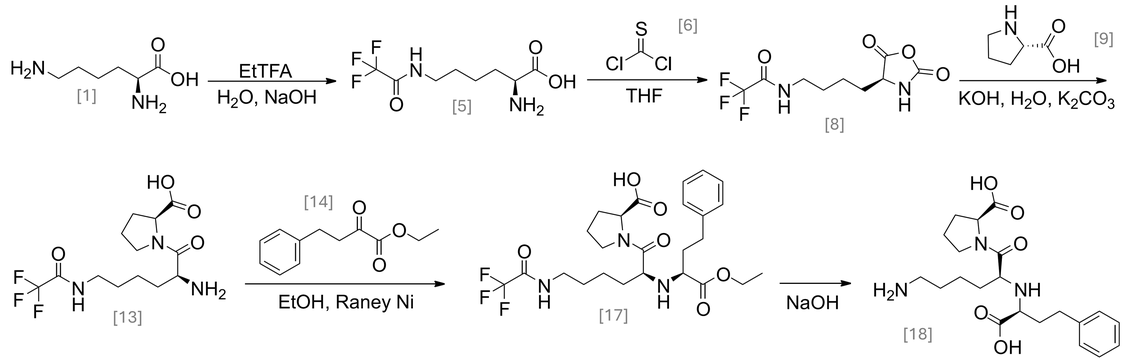


Methods

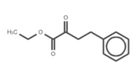
We constructed two (Q)SAR models, one statistical and one expert rule-based. The expert rule-based model consisted of 15 rules, covering both activating and deactivating features. The rule-based system was built into a nitrosation tool that also included a database of nearly 700 nitrosation reactions. After the model generates the probable nitrosamines, the database is searched for similar parent/nitrosamine pairs using a custom-built fingerprint.



Results: Reaction Schemes



Component	Description	Nitrosamines/ Scavengers/Catalysts
[1]	—	●
EtTFA	Ethyl Trifluoroacetate	
H2O	Water	
[4]	HO—Na	
[5]	—	●
[6]	Thiophosgene	
THF	Tetrahydrofuran	
[8]	—	●●
[9]	Proline	●

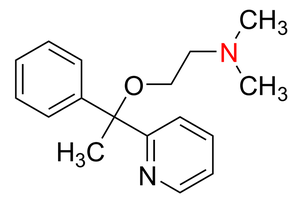
Component	Description	Nitrosamines/ Scavengers/Catalysts
[10]	HO—K	
K2CO3	Potassium Carbonate	
[K+]	Potassium hydride	
[13]	—	●
[14]		
EtOH	Ethanol	
Raney Ni	Raney Nickel	
[17]	—	●●
[18]	—	●

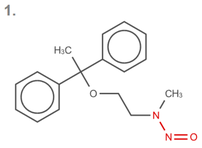
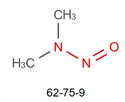
● Nitrosamines ● Nitrite Scavengers ● Nitrosation Catalysts

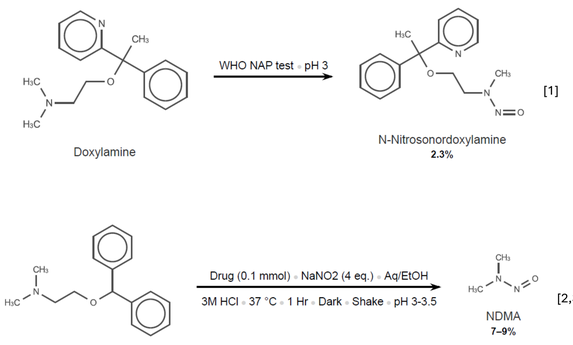
A user can also load their own reaction scheme into the tool to assess it for nitrosamine sources (●) using the expert rules. The tool will also recognize nitrite scavengers (●) and nitrosation catalysts (●). For the synthesis of Lisinopril (18), while there are multiple potential sources for nitrosamines, there is also a nitrite scavenger (1) at the beginning of the scheme. That scavenger can reduce the availability of reactive nitrite.

Results: Doxylamine

Doxylamine contains one tertiary amine and could theoretically produce one of two unique nitrosamines: *N*-Nitrosodoxylamine and NDMA. Both have a deactivating feature due to being derived from a tertiary amine (Score = -4). NDMA also has an additional deactivating feature due to having a leaving group larger than ethyl (Score = -4 + -2).



Nitrosamine	Nitrosation Likelihood	Carc. Study Data and Acceptable Intake (AI) Limits
1.  **Exact Mass:** 284.15248 Da	less likely Score = -4	**CPCA AI** 18 or 26.5 ng/day
2.  62-75-9 NDMA, N-Nitrosodimethylam... **Exact Mass:** 74.04801 Da	negligible Score = -6	**Carcinogenicity TD_{50}** 0.09 mg/kg/day Robust study data **CPCA AI** 18 or 26.5 ng/day **Regulatory AI** US FDA = 96 ng/day HC = 96 ng/day EMA = 96 ng/day



Features Used in Nitrosation Score Calculation

N-Nitrosodoxylamine

Deactivating (-4): Tertiary amine

Overall Score = -4 (less likely formation)

NDMA

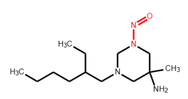
Deactivating (-2): Tertiary amine, leaving gp larger than ethyl
Deactivating (-4): Tertiary amine

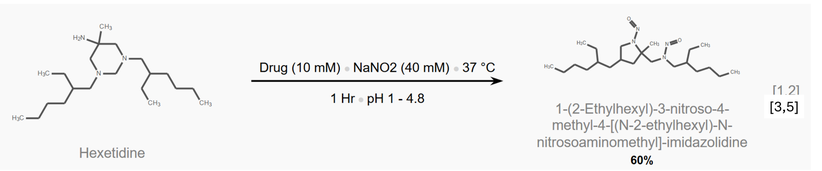
Overall Score = -6 (negligible formation)

The reaction database searches for both exact matches and for similar analogues based on a fingerprint of the reaction. The fingerprint not only records the reactant and product, but also which bonds need to be broken or formed to convert the parent amine into the predicted nitrosamine.

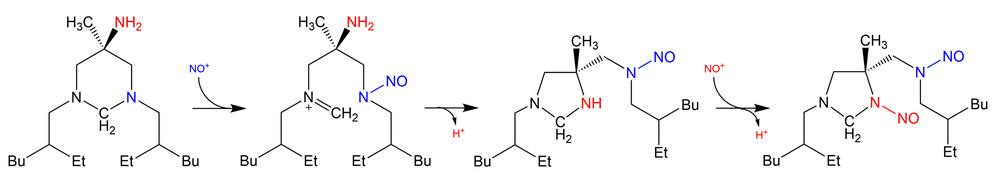
In the case of Doxylamine, only one exact match is found, that being the formation of the larger *N*-Nitrosodoxylamine. There are no exact reaction matches for NDMA. However, a similar hit is found, Diphenhydramine, which shares a very similar environment around the tertiary amine and has some evidence of NDMA formation.

Results: Hexetidine

Nitrosamine	Nitrosation Likelihood
 **Exact Mass:** 256.22631 Da	unlikely Score = -8



The database can also find nitrosamines that are produced by mechanisms not covered by the expert rules, such as the dually-nitrosated Hexetidine. In this case, the expert rules do not account for the opening of rings or the nitrosation of primary amines, but the database is able to find a reaction that does.



Conclusions

Both statistical and rule-based models, performed very well, however they could be improved with access to more experimental data. The reaction database has proved to be extremely useful in confirming the predicted nitrosamines and providing alternative mechanisms based on experimental data for similar compounds. Such results are extremely useful in providing a solid backing for the predictions made by the two (Q)SAR models. Additionally, the supplementary databases of nitrosation catalysts and scavengers provides an extra layer of information when performing risk assessment of specific synthetic routes or attempting to synthesize a nitrosamine for further risk analysis, such as the Enhanced Ames Test.

- Reiss, K., Saiakhov, R., & Chakravarti, S. (2025). *(Q)SAR Approaches to Predict the Extent of Nitrosation in Pharmaceutical Compounds*. **Chemical Research in Toxicology**, *Accepted*, DOI: 10.1021/acs.chemrestox.4c00435
1. Schmidtsdorff, S., Neumann, J., Schmidt, A. H., & Parr, M. K. (2022). *Risk Assessment for Nitrosated Pharmaceuticals: A Future Perspective in Drug Development*. **Archiv Der Pharmazie**, *355(4)*, 2100435.
2. Brambilla, G., Cajelli, E., Finollo, R., Maura, A., Pino, A., & Robbiano, L. (1985). *Formation of DNA-damaging nitroso compounds by interaction of drugs with nitrite. A preliminary screening for detecting potentially hazardous drugs*. **Journal of Toxicology and Environmental Health**, *15*, 1-24.
3. Brambilla, G. & Martelli, A. (2007). *Genotoxic and carcinogenic risk to humans of drug–nitrite interaction products*. **Mutation Research**, *635(1)*, 17-52.
4. A. Sakai, T. Inoue, A. Tanimura. (1984). *Formation of volatile nitrosamines by drug–nitrite interactions under physiological conditions*. **Gann**, *75*, 245-252.
5. Bae, J.Y., Mende, P., Shevlin, G., Spiegelhalder, B., Preussmann, R., & Loeppky, R.N. (1994). *The nitrosation of hexetidine and hexedine: characterization of the major nitrosamine from common antimicrobial agents*. **Chemical Research in Toxicology**, *7*, 868-876.