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

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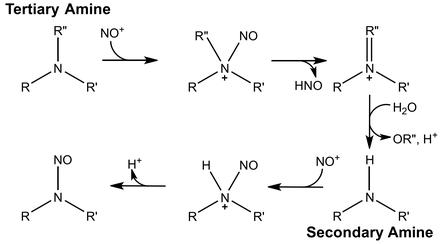
Background & Purpose

Since NDMA was discovered in pharmaceuticals in 2018, there has been a need for tools that can predict which compounds are likely to produce nitrosamines. *In-chemico* tests, such as the NAP test, do exist to predict how susceptible an amine is to nitrosation, but benchtop testing cannot match the efficiency of high-throughput *in-silico* workflows.

Our goal was to build a (Q)SAR model from the available experimental data to predict if (a) an amine is likely to be nitrosated and (b) what nitrosamines would be produced. We also sought to create a database of existing nitrosation reaction data to support the predictions made by the model through comparison with structurally similar compounds.

Tertiary Amine

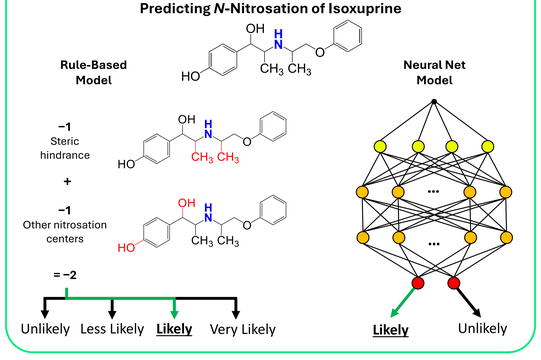


Methods

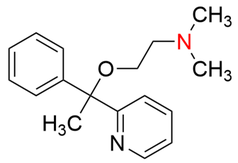
We constructed two (Q)SAR models, one statistical and one expert rule-based. Both were based on available nitrosation literature. The statistical model used graph convolution neural networks that were trained on NAP test results. The expert rule-based model consisted of 15 rules, covering both activating and deactivating features.

The rule-based system was built into a nitrosation tool that also included a database of nearly 700 nitrosation reactions. When a query molecule is submitted, the rule-based model generates the plausible nitrosamines for each amine, along with a likelihood score based on the expert rules. Then, the reaction database is searched for the most similar parent/nitrosamine pairs, including exact hits, if they exist.

Predicting *N*-Nitrosation of Isoxuprine



Results: Doxylamine

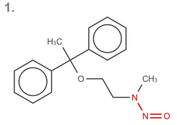
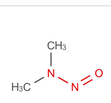


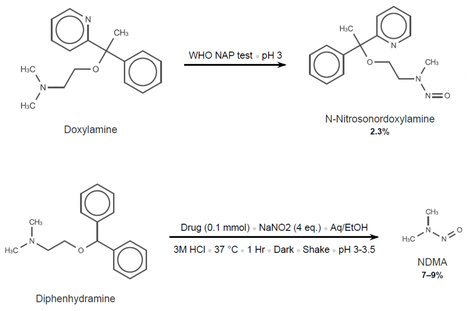
Doxylamine contains one tertiary amine and could theoretically produce one of two unique nitrosamines: *N*-Nitrosodoxylamine and NDMA. Both have a deactivating feature due to being derived from a tertiary amine. NDMA also has an additional deactivating feature due to its large leaving group.

Features Used in Nitrosation Score Calculation

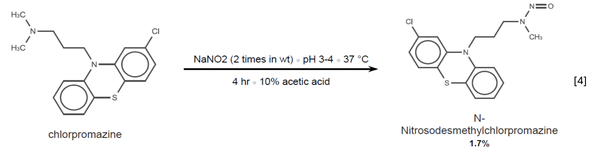
N-Nitrosodoxylamine
Deactivating (-4): Tertiary amine
Overall Score = -4 (less likely formation)

NDMA
Deactivating (-2): Tertiary amine, leaving gp larger than ethyl
Deactivating (-4): Tertiary amine
Overall Score = -6 (negligible formation)

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

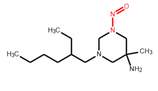


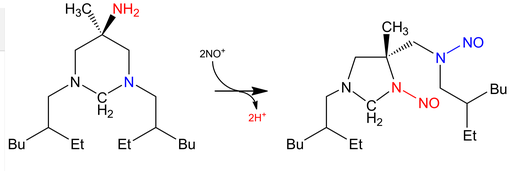
The reaction database searches for both exact matches and for similar analogues based on a fingerprint of the reaction. The fingerprint not only records the reactant and product, but also which bonds need to be broken or formed to convert the parent amine into the predicted nitrosamine.

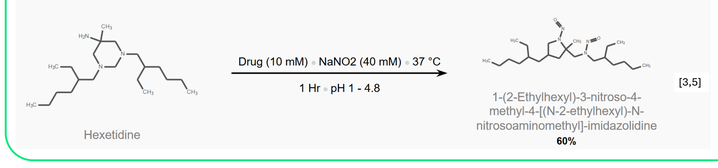


In the case of Doxylamine, only one exact match is found, that being the formation of the larger *N*-Nitrosodoxylamine. There are no exact reaction matches for NDMA. However, a similar hit is found, Diphenhydramine, which shares a very similar environment around the tertiary amine and produces NDMA.

Results: Hexetidine

Nitrosamine	Nitrosation Likelihood	Carc. Study Data and Acceptable Intake (AI) Limits
1.  **Exact Mass:** 256.22631 Da	unlikely Score = -8	**CPCA AI** 400 ng/day

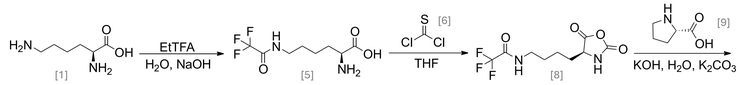


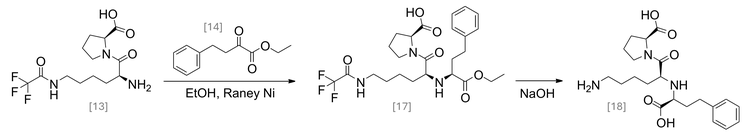


The database can also find nitrosamines that are produced by mechanisms not covered by the expert rules, such as the dually-nitrosated Hexetidine. In this case, the expert rules do not account for the opening of rings or the nitrosation of primary amines, but the database is able to find a reaction that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

Results: Reaction Schemes

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 will help to minimize the amount of reactive nitrite.





Component	Description	Nitrosamines/ Scavengers/C atalysts
[1]	—	●
EtTFA	Ethyl Trifluoroacetat e	
H2O	Water	
[4]	HO — Na	
[5]	—	●
[6]	Thiophosgene	
THF	Tetrahydrofuran	
[8]	—	●●
[9]	Proline	●
[10]	HO — K	
K2CO3	Potassium Carbonat e	
[K+]	Potassium hydride	
[13]	—	●
[14]		
EtOH	Ethanol	
Raney Ni	Raney Nickel	
[17]	—	●●
[18]	—	●

● Nitrosamines ● Nitrite Scavengers ● Nitrosation Catalysts

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.

For more details on the statistical and expert rule-based models, please use the QR code below to check out our paper, which was recently accepted by *Chemical Research in Toxicology* and is available as an open access article.

❑ 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
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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.
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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.