A Comprehensive Set of Structural Keys for *N*-Nitrosamine Fingerprinting and Determining Surrogate Relevance in Carcinogenic Potency Assessments

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Purpose

- Identify structural features for efficient searching and quantitative comparison of nitrosamine surrogates.
- Identify surrogates that mimic the reactivity and structure of complex Nitrosamine Drug Substance Related Impurities (NDSRIs).
- Identify features that affect CYP-450 mediated α-hydroxylation of nitrosamines and their carcinogenic potency.

Methods

Feature Sets

We assembled three structural feature sets relevant to N-nitrosamines' carcinogenic potency:

1. CPCA[1,2] Based Structural Keys (FP-1)

- Various combinations of α-H counts.
- Carboxylic group anywhere.
- Various types of ring memberships.
- Various activating and deactivating features.
- Calculated activating and deactivating scores
- Fingerprint length = 33 bits.

2. Atom Types Around the N-N=O Moiety[3] (FP-2)

- Atoms up to 5 bond distance were used.
- Different atom types were counted at each bond depth.
- Atom types were defined using element type, hybridization, H counts, aromaticity, ring membership etc. – total 193 atom types.
- Fingerprint length = 990 bits, consists of 5 equal segments.

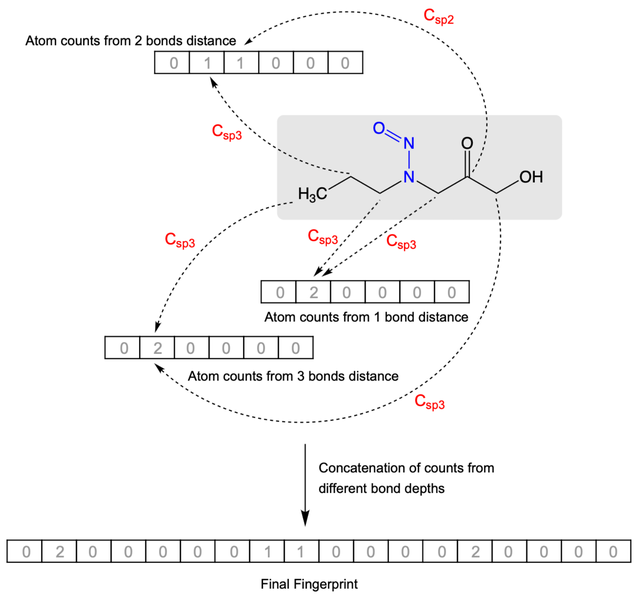


Figure 1. Generating a fingerprint using the counts of various atom types around the nitrosamine moiety

3. Metabolic α-CH_2 Hydroxylation Modulators[4]

- Consists of activating and deactivating structural features that influence α-carbon hydroxylation.
- Identified using QSAR modeling of α-carbon hydroxylation in xenobiotic CYP metabolism.

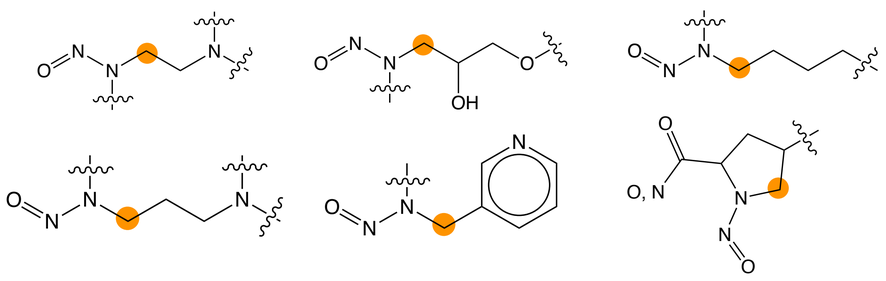


Figure 2. Examples of some deactivating features (i.e., inhibits α-CH_2 hydroxylation) identified from QSAR modeling

Data

- A set of 209 small nitrosamines with available animal carcinogenicity data were used as surrogates.
- 620 pairs of xenobiotic substrate-metabolite pairs were used to build the α-CH_2 hydroxylation QSAR models.

Similarity Measure

- For FP-1, Tanimoto similarity was used.
- For FP-2, the fraction of atom type and ring membership matches between two nitrosamines were used as the similarity measure.

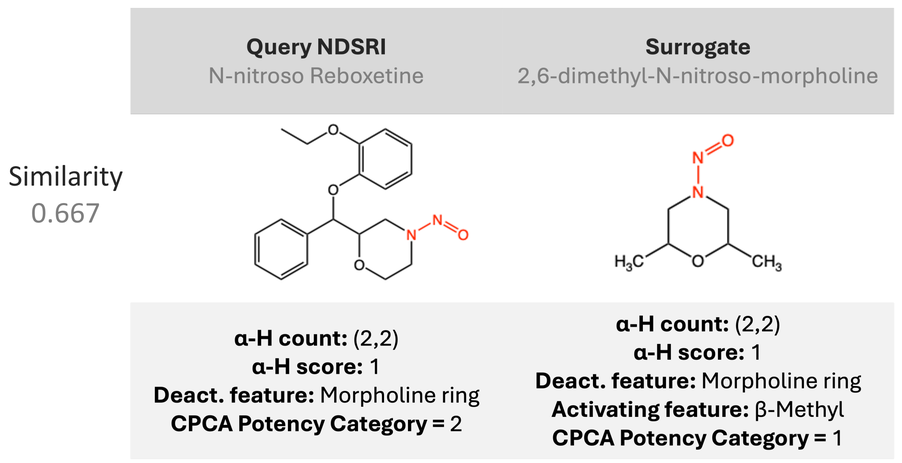
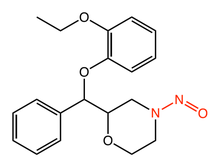


Figure 3. Computing similarity between an NDSRI and a nitrosamine surrogate using CPCA-based structural keys (FP-1)

Results



Query NDSRI (N-nitroso Reboxetine)
EMA AI = 127 ng/day

Surrogate	TD_{50} (mg/kg/day)	Robustness of Carcinogenicity Study	Regulatory AI (ng/day)	Predicted CPCA AI (ng/day)	α-H Count & Score	Feature Scores
Sim. = 1.000 59-89-2 NMOR, N-nitroso-morpholin...	0.12	★★★	HC = 127 EMA = 127	100	(2, 2) Score = 1	Deact. = 1 Act. = 0
Sim. = 0.667 1456-28-6 2,6-dimethyl-N-nitroso-mo...	1.22	★	-	18 or 26.5	(2, 2) Score = 1	Deact. = 1 Act. = -1
Sim. = 0.600 13147-25-6 N-Ethyl-N-(2-hydroxyethyl...	0.02	★★	-	100	(2, 2) Score = 1	Deact. = 1 Act. = 0

Table 1. Identified top surrogates for the NDSRI using the CPCA based fingerprint (FP-1)

Registry Number	Chemical	Similarity	Name	Mol. Wt.	CPDB TD50 (mg/kg/day)
34993-08-3		0.666	3-Methyl-4-nitroso-2-phenylmorpholine	206.245	10000
55557-03-4		0.644	N-Nitroso-methylphenidate	262.309	10000
1456-28-6		0.575	2,6-dimethyl-N-nitroso-morpholine	144.174	1.22

Table 2. Identified top surrogates for the NDSRI using the general atom type-based fingerprint (FP-2)

Conclusions

- Expert visual assessment confirmed the fingerprints' effectiveness in identifying suitable surrogates for NDSRI intake limits.
- CPCA feature-based fingerprints (FP-1) outperform traditional atom type fingerprints (FP-2).
- Fingerprints offer a quantitative measure of nitrosamine similarity, relevant for their carcinogenic potency.

References

[1] US FDA; Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry. 2023; [2] EMA. Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products; [3] Kruhlak et al., A New Structural Similarity Method to Identify Surrogate Compounds for Assessing the Carcinogenicity of Nitrosamine Impurities, SOT Annual Meeting, San Diego, Poster, 2022; [4] Chakravarti, S.K., Computational Prediction of Metabolic α-Carbon Hydroxylation Potential of N -Nitrosamines: Overcoming Data Limitations for Carcinogenicity Assessment. Chem. Res. Toxicol. 2023, 36, 959–970.