Collaborative analysis of complex nitrosamines

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Introduction

The recent discovery of small-molecule nitrosamine impurities in marketed drugs, starting with nitrosodimethylamine (NDMA) in batches of Valsartan in 2018, has led to significant regulatory response, including drug recalls and regulatory guidance that requires the evaluation of all synthetic and formulation routes for the potential presence of nitrosamine impurities. Due to the wide range of potential routes of formation for nitrosamines[1], many active pharmaceutical ingredient (API) structures are themselves liable to be nitrosated, either during the later stages of the synthetic process or as the formulated drug product.

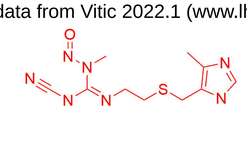
This poster describes the formation of a data-sharing initiative, coordinated by Lhasa Limited, which has the following aims:

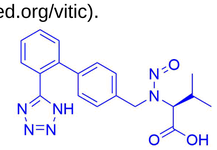
- To reduce duplication of testing and make available Ames test results conducted to the highest standards and in appropriate conditions, which will reduce potential uncertainties for regulatory submissions consequently.
- To investigate differences between these compounds and the small molecules which comprise the majority of publicly available nitrosamine data[2] and elucidate any structure activity trends and significant differences.

Key among these is that based on initial reports and the small amount of data for complex structures that is currently available, the proportion of these compounds which are Ames negative may be higher than the proportion of small molecule nitrosamines, for reasons such as differences in metabolic potential or the proposed adducts formed. Full testing of this hypothesis requires the curation of a large high-quality dataset, which can only be achieved via cross-industry collaborations such as this.

Existing data landscape

Ames data for a small number of structurally complex nitrosamines is available; two examples are shown below (red = Ames positive – yet carcinogenicity negative, showing the complexity of the problem, blue = 5-strain negative; data from Vitic 2022.1 (www.lhasalimited.org/vitic).

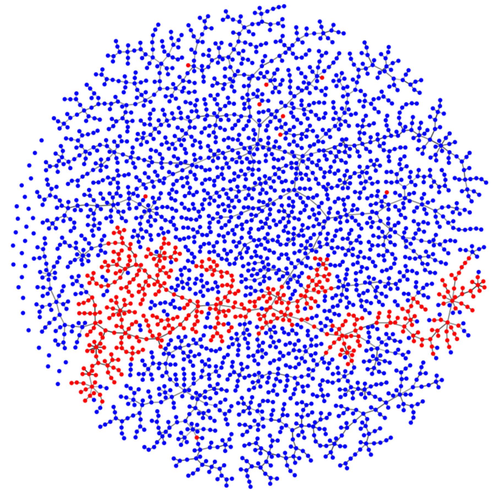




Previous work in this area[3,4] has focused on the nitrosation assay protocol (NAP) test, where compounds are subjected to strongly-nitrosating conditions in what is essentially a forced degradation experiment. While an understanding of degradation is undeniably important, the forcing conditions lead to the nitrosation of a variety of potential sites, not just amines – and indeed the degradation of the target to release a small secondary amine such as dimethylamine which is then nitrosated. An Ames positive for the reaction mixture generated does not necessarily indicate that the complex nitrosamine itself will be positive, especially if the identification of the nitrosamine is restricted to seeking a +29 *m/z* peak.

"Small" nitrosamines

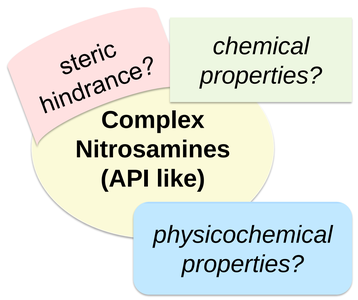
The majority of toxicity data that exists is for small-molecule nitrosamines, with nitrosodiethylamine and nitrosodimethylamine the most studied as well as among the most potent. This makes extrapolation from this data potentially challenging, since the chemical space covered by the available toxicity data is clearly different – the nine nitrosamines distributed within the drug data are arguably the only druglike ones.



The figure shows a Minimum Spanning Tree (a subset of the fully connected similarity graph, with each compound connected to its single most similar neighbour) generated in Lhasa's cheminformatics toolkit, based on the ECFP4 fingerprint. Red indicates the nitrosamines for which toxicity data is available in Vitic (version 2022.1, Lhasa Limited, www.lhasalimited.org/vitic), blue currently marketed drugs (ChEMBL 29, https://www.ebi.ac.uk/chembl/).

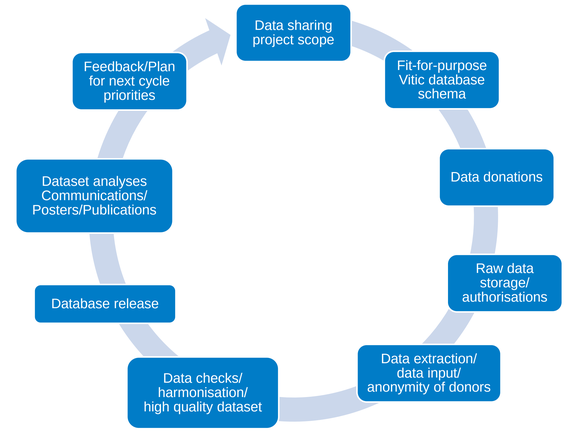
Expected differences

Anecdotal reports suggest that a much higher proportion of complex nitrosamines are Ames-negative than the <20% of small-molecule nitrosamines that are Ames negative[1]. Drug molecules have been engineered for precise activity and DMPK profiles, which may negate the effects of the nitroso group. The bulkier drug molecules may lead to different adducts, but also may affect α-hydroxylation of the nitrosamine, and other functionality on the drug may affect the preferred site of metabolism.



Data-sharing collaborations

Extensive testing of complex nitrosamines – synthesized on purpose and fully characterized – is currently underway as part of the second stage of the regulatory response to the current crisis. The need for large numbers of Ames tests, and potential *in vivo* follow-up assays, leads to pressure on the few laboratories able to carry out these tests. As a result, the sharing of testing results amongst companies – both discovery pharmaceutical companies and generic manufacturers, leads to a reduction in the testing burden for each member.



How is the data going to be used?

However, the simple sharing of data is not the sole purpose of this initiative. Rather, the main aim of the group is to share data on structurally complex nitrosamines, investigate the hypotheses given at left – are complex nitrosamines actually different in their activity to small dialkyl nitrosamines?

- Confidential sharing of data
- Comparison of Ames results
  - Different outcomes
  - Different strain profiles
- SAR investigation of differences
- Publish analysis for all to benefit

References

1. Lopez-Rodriguez *et al* (2020), *Org. Process Res. Dev*. **24**, 1558-1585.
2. Thresher *et al* (2020), *Regul. Toxicol. Pharmacol.,* **116**, 104749.
3. Brambilla and Martelli (2007), *Mutat. Res.,* **635**, 17-52.
4. Schmitsdorff *et al* (2022), *Arch. Pharm*. e2100435.