In silico prediction of N-nitrosamine degradants in API's that possess a secondary or tertiary amine functional group

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Introduction

N-Nitrosamines in pharmaceutical drug substances and products are regarded as a cohort of concern as they are considered potential human carcinogens (Figure 1)[1]. Their levels as impurities is guided by the ICH M7 guidance and has led to recall of marketed drug compounds such as ranitidine and sartan medicines[2]. The industry guidance issued by the FDA (2021 revision) stipulates that a risk assessment of the degradation pathway of a drug product should be considered to assess the risk of nitrosamine impurity formation[3] (Scheme 1).

Drug degradation knowledge can be encapsulated within a knowledge base as a collection of transformations, accessible through the *in silico* tool Zeneth[4]. Recently a series of transformations predicting the N-nitrosation of secondary and tertiary amines have been developed. Their general chemical space and mechanism is discussed, together with the environmental conditions under which they are deployed (as a prediction) for a chemically relevant API.

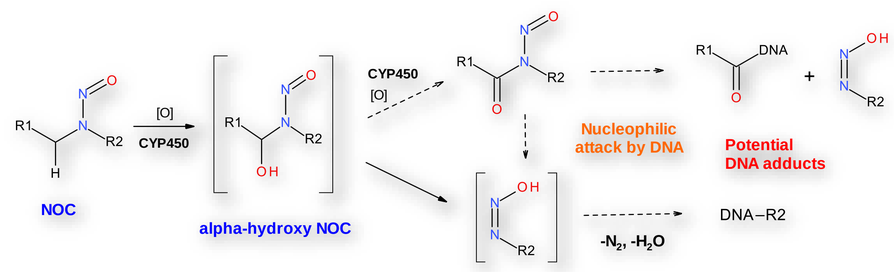
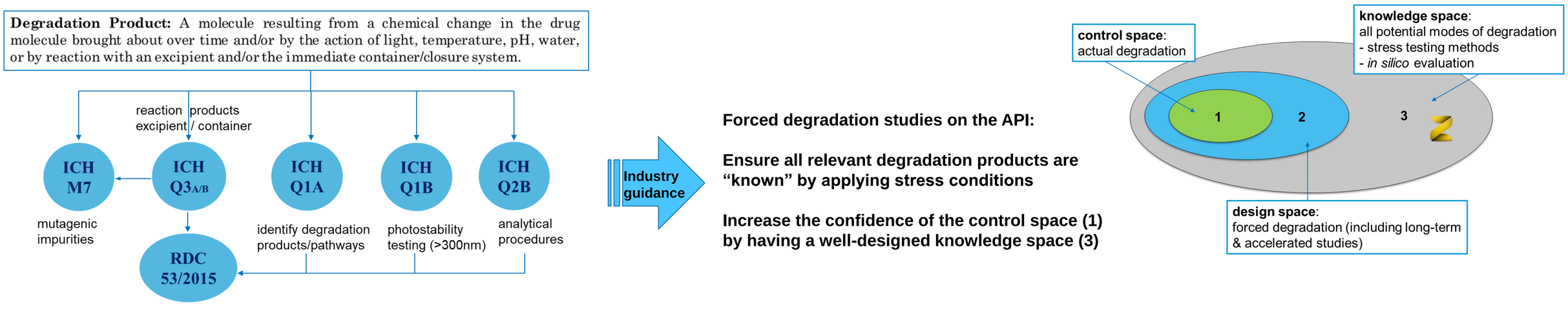


Figure 1: Cytochrome P450-catalysed metabolism of NOCs producing DNA-damaging intermediates.



Scheme 1: International regulatory guidance of a new degradation product (including types of studies to be conducted and characterisation of the degradation products).

Formation of N-nitroso compounds

Nitrites (NO_2^-) and nitrates (NO_3^-) can react with secondary and tertiary amines to generate N-nitroso compounds (NOCs)[5]. Many API's contain secondary/tertiary amine functionality and if contaminated with a source of nitrites/nitrates (from excipients or container/packaging) then a nitrosation reaction could take place.

A nitrosating agent is regarded as a nitrosonium ion carrier ([NO^+]), of electrophilic character, which is prone to attack nucleophiles such as amino compounds[6]. The most general nitrosating reagent is nitrous acid formed by reaction of a mineral acid, with sodium nitrite in water or in a mixture of water and an organic solvent (Figure 2).

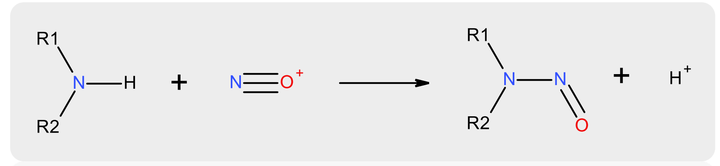
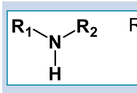


Figure 2: N-Nitrosation of a secondary amine by [NO+] carrier.

Results and discussion

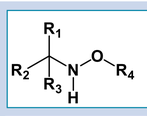
In order to evaluate the chemistry that should be covered by the Zeneth knowledge base, a thorough investigation of the literature was conducted and a total of 8 new transformations have been scoped, covering both secondary and tertiary amines:

Classes of secondary amines (and analogues)



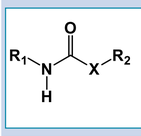
R1, R2 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon

Secondary amines



R1 = aliphatic carbon or hydrogen
R2, R3 = aliphatic carbon, aromatic carbon or hydrogen
R4 = aliphatic carbon (not multiply bonded or attached to another heteroatom), aromatic carbon or hydrogen

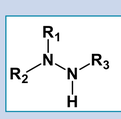
Hydroxylamines



X = C(R3)-R4, N-R5, O or S (divalent)
R1, R2 = aliphatic carbon (not multiply bonded) or aromatic carbon
R3-R5 = aliphatic carbon (not multiply bonded), aromatic carbon or hydrogen

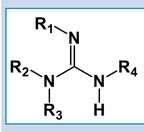
R2 may also be hydrogen in the case of amides.

Secondary amides, carbamates, ureas



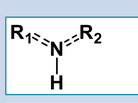
R1= aliphatic carbon or aromatic carbon
R2 = aliphatic carbon, aromatic carbon or hydrogen
R3 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon

Hydrazines



R1 = any atom
R2= aliphatic carbon or aromatic carbon
R3 = aliphatic carbon, aromatic carbon or hydrogen
R4 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon

Guanidines



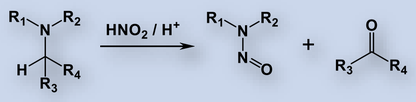
R1, R2 = carbon or nitrogen

The bonds R1-N-R2 must be aromatic.

Aromatic nitrogen (pyrrole)

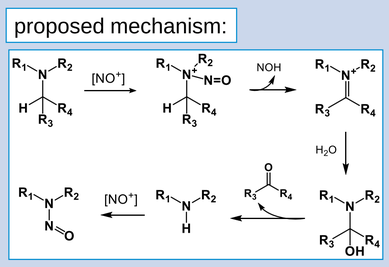
Classes of tertiary amines

1. Aliphatic type bearing an alpha hydrogen and undergoing an N-dealkylation step[5]:

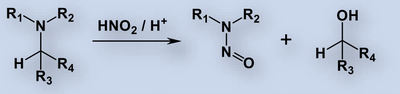


R1, R2 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon
R3, R4 = aliphatic carbon (not multiply bonded or attached to a heteroatom) or hydrogen

proposed mechanism:



2. Benzylic type (beta carbon is aromatic)[7]:



R1, R2 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon
R3 = aliphatic carbon (not multiply bonded or attached to a heteroatom) or hydrogen
R4 = aromatic carbon

proposed mechanism:

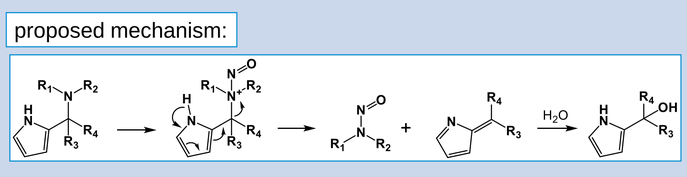


Table 1: New transformation classes for secondary and tertiary amines (nitrosation taking place with nitrous acid under acidic conditions).

Conclusions

In total, eight transformations have been developed and are currently under review to be incorporated into the Zeneth knowledge base. Generally, tertiary amines are much less reactive than secondary amines because they require an additional dealkylation step (Table 1); the α-CH bond breakage deemed to be the rate determining step. Hence these will have a lower likelihood score in Zeneth. The addition of this chemistry now allows Zeneth to predict the formation of NOCs observed for compounds such as norfloxacin, ranitidine, doxylamine and metformin, when the presence of nitrite/nitrous acid is indicated.

References

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