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Prediction of Drug Degradation Pathways leading to Structural Alerts for Potential Genotoxic Impurities

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Abstract:

An in-depth analysis of the web-based CambridgeSoft Pharmaceutical Drug Degradation Database, Pharma D3, was conducted in two phases in an attempt to generate some general rules for the prediction of alerting structures for genotoxicity that may arise as a result of degradation. The first phase involved interrogation of the database to determine the nature and frequency of alerting structures present in the degradants. This analysis revealed five functional groups, which account for approximately 70% of the alerting structures found in the degradants within the database: (1) aldehydes; (2) α,β unsaturated carbonyls; (3) aromatic amines, hydroxylamine and its derived esters; (4) epoxides; and (5) polyaromatic hydrocarbons. The second phase of the analysis involved categorizing the major chemical reactions responsible for the generation of the five most prevalent alerting structures. This two-step approach led, in turn, to a proposal for the prediction of functional groups that may have a propensity to degrade to alerting structures not necessarily present in the parent molecule.

1. Introduction

Strategies for dealing with genotoxic impurities or potential genotoxic impurities arising from drug synthesis have received considerable attention in the literature in recent years.[1−6] In contrast to process impurities, genotoxic degradants have received less attention but, in fact, require special consideration since there is no opportunity for purification and their presence needs to be considered over the entire shelf life of the product. The fact that genotoxic or potentially genotoxic impurities can arise from degradation of the active ingredient in both the drug substance and the drug product adds an additional element of complexity compared with the control of process impurities.

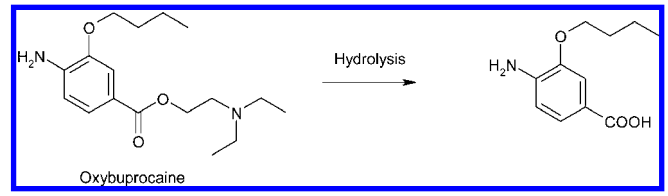
If drug degradation does indeed lead to the presence of a genotoxin, only a very small degree of degradation is necessary to produce levels above the acceptable thresholds.[1,2] Furthermore, the level of a genotoxic impurity may have to be controlled below the threshold of toxicological concern (TTC) level in the drug substance to ensure that it does not exceed the TTC level in the drug product over its shelf life. Therefore, an understanding of the potential for genotoxins to be formed *via* drug degradation is crucial to the development of adequate control strategies. A systematic approach to the prediction of potential genotoxic degradants should be a valuable tool in support of effective and efficient drug development. This article focuses on elucidating and classifying the typical and most common mechanisms of formation found for alerting structures in degradants of typical drug molecules. This effort is based on a thorough evaluation of published information by correlating structures of a wide variety of drugs and related degradants.

2. Origins for Structural Alerts in Potential Genotoxic Degradants

Conceptually, there are two main ways in which an alerting genotoxic drug degradant structure can form.

2.1. Parent Drug That Already Contains a Genotoxic Alert. The parent drug that already contains a genotoxic alert forms a degradant (a) in which the original alerting structure is conserved or (b) a different alerting structure is produced. Two examples follow to illustrate this concept.

2.1.1. Degradant with Same Alerting Structure As the Parent Drug.



In this case, oxybuprocaine (trade name: Novesin) with a structural alert for aromatic amines forms the corresponding

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(1) *Guideline on the Limits of Genotoxic Impurities*, CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006; Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency (EMEA): London, 28 June 2006.
(2) *Questions and answers on the CHMP guideline on the limits of genotoxic impurities*,; Committee for Medicinal Products for Human Use Safety Working Party(CHMP), European Medicines Agency (EMEA): London 2008.
(3) Genotoxic and carcinogenic impurities in drug substances and drug products: Recommended approaches, Guidance for Industry (draft); U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, 2008; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079235.pdf.
(4) Müller, L.; Mauthe, R. J.; Riley, C. M.; Andino, M. M.; Beels, C.; DeGeorge, J.; De Knaep, A. G. M.; Ellison, D.; Fagerland, J. A.; Frank, R.; Fritschel, B.; Galloway, S.; Harpur, E.; Humfrey, C. D. N.; Jack, A. S.; Jagota, N.; MacKinnon, J.; Mohan, G.; Ness, D.; O'Donovan, M. R.; Smith, M. D.; Vudathala, G. K.; Yotti, L. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Reg. Toxicol. Pharmacol.* **2006**, *44*, 198–211.
(5) Pierson, D. Approaches to assessment testing decisions and analytical determination of genotoxic impurities in drug substances. *Org. Process Res. Dev.* **2009**, *13* (2), 285–291.
(6) Snodin, D.; Vudathala, G. K. Genotoxic impurities: A case for regulatory rethink. *AAPS Mag.* **2009**, (Feb), 20–26.

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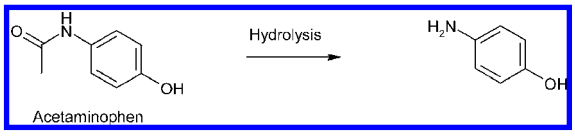
Table 1. **Toxtree evaluation of the CambridgeSoft D3 drug degradant database showing the number of hits for structural alerts in the parent drug and in the corresponding degradant**

structural alert and corresponding ToxTree alert number	number of hits in parents	number of hits in degradants	number of unique hits in degradants[a]
alkyl or benzyl ester of sulfonic or phosphonic acids	3	2	0
N-methylol derivatives	0	2	2
monohaloalkenes	0	3	3
propiolactones and propiosultones	4	5	4
epoxides and aziridines[b]	**9**	**17**	**12**
aliphatic halogens	12	12	6
α,β-unsaturated carbonyls[b,c]	**79**	**126**	**30**
aldehydes[b]	**2**	**40**	**34**
quinones	11	23	4
hydrazines	11	12	5
aliphatic azos and azoxys	0	1	1
alkyl carbamates and thiocarbamates	5	18	0
polycyclic aromatic hydrocarbons	0	6	6
heterocyclic,polycyclic aromatic hydrocarbons[b]	**4**	**15**	**13**
azide and triazene groups	7	2	0
α,β-unsaturated alkoxy	0	1	1
aromatic nitroso groups	0	2	2
aromatic ring *N*-oxides	0	6	6
nitro aromatics	26	25	6
primary aromatic amines, hydroxyl amines and its derived esters[b]	**73**	**93**	**23**
aromatic monoand dialkylamines	3	4	2
aromatic *N*-acyl amines	5	8	5
aromatic diazos	0	2	2
coumarins and furocoumarins	0	1	1

[a] Unique hit in Degradant means that the alerting structure is not shared with the parent drug. [b] The degradants shown in **bold** collectively represent approximately 70% of the positive hits for all degradants in the database. [c] The Toxtree analysis of quinones fires a positive hit response for this class, as quinones contain the α,β-unsatured carbonyl structural element. In this table, the numbers reported for the α,β-unsatured carbonyl class includes the hits for quinones.

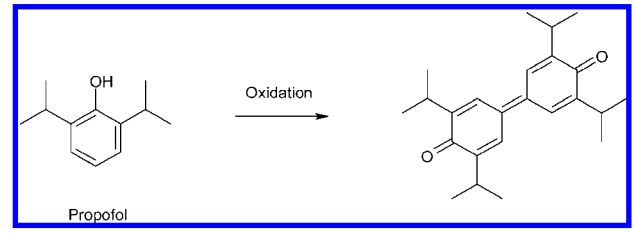
acid *via* hydrolysis, and the structural alert for aromatic amines is retained in the degradant.[7] According to EMEA guidance and further described by Dobo et al.,[8] impurities (or in this case degradants) with structural alerts that are shared by the parent molecule can be qualified by standardized mutagenicity data obtained with the parent molecule. In addition, however, the chemical constraints for the alerting structure should be similar for both the parent molecule and degradant such that reactivity of the alerting species should not be significantly different. In contrast, a degradant that has a unique alerting structure is not considered qualified with the drug substance.

2.1.2. A Degradant with a Different Alerting Structure than Parent Drug Is Formed.



In this case, acetaminophen (trade name: Tylenol), which itself contains a structural alert for *N*-acylated aminoaryls, forms *p*-aminophenol.[9] This triggers a structural alert for an aromatic amine, which is different from the alert in the parent structure.

2.2. Parent Drug with No Alerting Structure. The parent drug with no alerting structure forms a degradant containing an alerting structure.



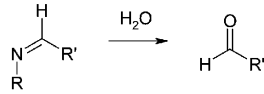
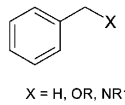
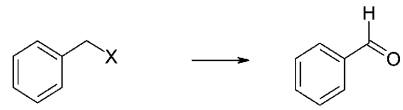
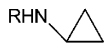
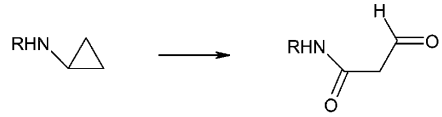
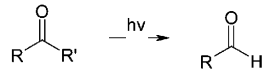
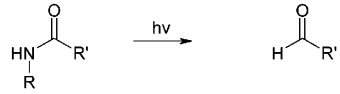
In this example, propofol (trade name: Diprivan), which lacks a structural alert, degrades *via* oxidation to a dimeric degradation product containing several conjugated unsaturated carbonyl systems, which are structural alerts for mutagenicity.[10]

3. Evaluation of the CambridgeSoft Pharma D3 Drug Degradant Database for the Presence of Genotoxic Structural Alerts

The CambridgeSoft Pharmaceutical Drug Degradation Database, Pharma D3, is a web-based, searchable database of drug substances and their respective degradation products (http://d3.cambridgesoft.com/), populated with information from various sources in the literature. The database can be searched by substructure, compound number, commercial name, formula and molecular weight for parent compounds or degradants or experimental conditions. (It is important to note that Pharma D3 does not distinguish between major and minor degradants.)

(7) El-Grindy, A. First derivative spectroscopy and LC determination of benoxinate hydrochloride and its degradation Products. *J. Pharm. Biomed. Anal.* **2000**, *22*, 215–234.
(8) Dobo, K. L.; Greene, N; Cyr, M. O.; Caron, S; Ku, W. W. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. *Regul. Toxicol. Pharmacol.* **2006**, *44* (3, April), 282–293.
(9) Florey, K., Ed. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1974; Vol. 3, p 39.
(10) Baker, M. T.; Gregroson, M. S.; Martin, S. M.; Buettner, G. R. *Crit. Care Med.* **2003**, *31* (3), 787–792.

Table 2. **Reactions leading to the production of an alerting structure: aldehydes**

Functional group in parent leading to degradation	**Mechanism** (in parenthesis are listed the names of the corresponding drugs in the Pharma D3 database exhibiting this degradation pathway)
Imine 	Hydrolysis (Nitrofurantoin) 
Benzylylalcohols, Benzylamines, Benzylic carbons:  X = H, OR, NR1R2	Oxidation (Albuterol, Chloroamphenicol, Econazole, Ezlopitant, Ibuprofen, Losartan, Miconazole) 
Amino-cyclopropyl 	Photo-oxidation (Cryomazine) 
Aromatic acetic acid 	Oxidation + decarboxylation (Indomethacine) 
Ketone 	Norrish Reaction (Methadone) 
Amide  R' = alkyl	Photolysis (Flutamide) 

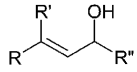
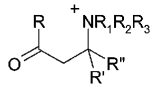
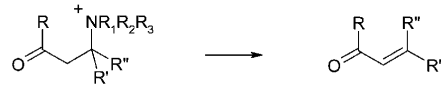
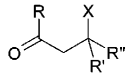
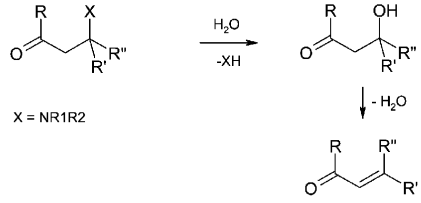
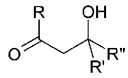
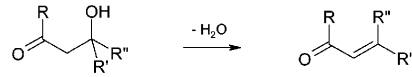
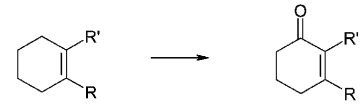
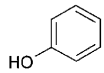
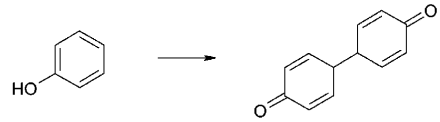
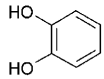
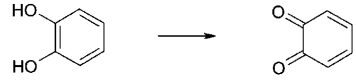
As of November 2009, Pharma D3 contained 322 unique parent structures and 1021 unique degradants.[11] We have analyzed the parent compounds and their respective degradant structures using Toxtree (v1.51) for the presence of alerting structures that are either unique to the degradant or shared with the parent. Toxtree is open-source software, which can be downloaded from the European Commission's Joint Research Centre (http://ecb.jrc.ec.europa.eu/qsar/). The software can be used to predict the toxic hazards of chemicals. Included within Toxtree is a decision tree for the prediction of *Salmonella* mutagenicity and carcinogenicity.[11] The decision tree contains a collection of rules, which is in essence a list of structural alerts for mutagenicity. Whereas structures may be analyzed by visual inspection, the collection of rules in Toxtree can serve as a more efficient knowledge base for the identification of potential mutagenic substances. (It should be noted that Toxtree searches for the presence of alerting structures without regard to the position of the altering structure in the molecule. Thus, ToxTree is a useful tool in defining the frequency with which structural alerts occur in degradation products. More sophisticated programs, such as MultiCase and DEREK, take into account not only the presence of an altering structure but also apply more sophisticated rules and machine learning.) The Pharma D3 database was found to contain the following numbers and types of alerting structures (see Table 1):

- 221 alerting structures among the 322 parent molecules (69%)
- 336 alerting structures among the 1021 degradants (33%)
- 155 alerting unique structures among the 1021 degradants (15%).

On the basis of these data, we identified and then classified the most common degradation reactions in the Pharma D3 database that form alerting structures not present in the parent molecule (i.e., unique structures). This analysis, (Table 1) showed that five functional groups accounted for almost 70% of the unique alerting structures. Tables 2−6 detail the general chemical reactions responsible for the production of those almost 70% of unique alerting structures. The focus of our effort was

(11) Benigni, R.; Bossa, C. Structure alerts for carcinogenicity, and the Salmonella assay system: A novel insight through the chemical relational databases technology. *Mutat. Res.* **2008**, *659* (3, Sep-Oct), 248–261.

Table 3. **Reactions leading to the production of an alerting structure: α,β-unsaturated carbonyls**

Functional group in parent leading to degradation	**Mechanism** (in parenthesis are listed the names of the corresponding drugs in the Pharma D3 database exhibiting this degradation pathway)
Allylalcohol 	Oxidation (Docetaxel) 
Quarternary ammonium group beta to carbonyl 	Hofmann elimination (Cisatracurium) 
Heteroatom β to carbonyl 	Substitution/elimination reaction induced with Water (Dyclonine) 
β-Hydroxycarbonyl 	Elimination of water (Streptovitacine) 
Cyclohexene 	Oxidation α to double bond (Cyclobarbital) 
Phenol 	Oxidative dimerization to quinone (Propofol) 
Catechol 	Oxidation to quinone (Adrenaline, Isoprenaline) 

to seek out reaction mechanisms thought to be general in nature and therefore applicable to related drug structures. The emphasis was on trying to find common themes that should be helpful for analyzing related drug structures and not to be exhaustive in listing every mechanism responsible for degradation to unique alerts in the D3 database. This approach should therefore allow Tables 2−6 to serve as a helpful tool for supporting the identification of functional groups in drug molecules that may lead to potentially genotoxic degradants.

The following abbreviations for substituents were used, except where noted otherwise in the tables: R, R′, R″, R1, R2, R3 = H or CH_x or C-alkyl or C-heteroatom. For aromatic structures that are drawn without substituents, the same degradation reaction would be expected to occur in the case of substituted versions, unless the type of substituent on the aromatic ring makes the degradation reaction chemically unlikely.

Table 4. **Reactions leading to the production of an alerting structure: primary aromatic amine, hydroxylamine, and its derived esters**

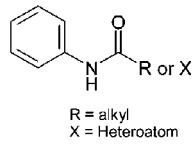
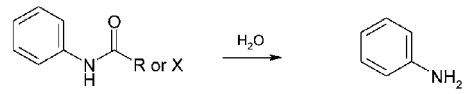
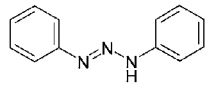
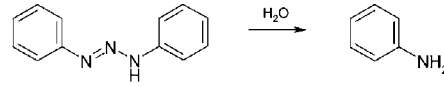
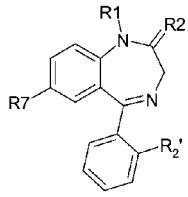
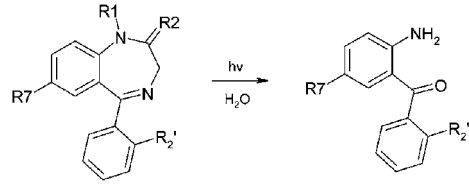
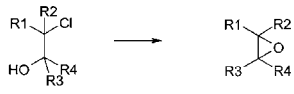
Functional group in parent leading to degradation	**Mechanism** (in parenthesis are listed the names of the corresponding drugs in the Pharma D3 database exhibiting this degradation pathway)
Aromatic N-acyl  R = alkyl X = Heteroatom	Hydrolysis (Acebutolol, Acetaminophen, Bicalutamide, Cinalukast, Clanfenur, Imatinib) 
Phenyltriazene 	Hydrolysis (Diminazene) 
Benzodiazepine  R1, R2, R2', R7 refer to the Benzodiazepine substitution nomenclature	Photoinduced Hydrolysis (Midazolam) 

Table 5. **Reactions leading to the production of an alerting structure: epoxides**

Functional group in parent leading to degradation	**Mechanism** (in parenthesis are listed the names of the corresponding drugs in the Pharma D3 database exhibiting this degradation pathway)
Double bond 	Oxidation (Indolizine, Menadione, Tanespimycin) 
2-hydroxy-1-chloroalkane 	Cyclization via Nucleophilic substitution (Mometasone, Ornidazole) 

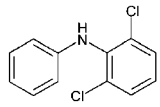
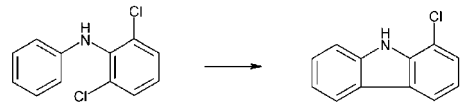
4. Discussion

Because the Pharma D3 database contains only a limited number of drugs, it is evident that Tables 2−6 contain just a subset of all the chemical reactions that can be expected to be relevant for the formation of alerting drug degradants formed from the corresponding parent drugs. Nevertheless, the fact that the tables are derived from a compilation of actual drugs (or drug-like compounds) and related degradation products should make them a valuable tool for the practitioner, as many of the known drug molecules to date contain recurring molecular themes.

It follows from the preceding discussion that the initial evaluation of a new drug substance for the possibility to produce potentially genotoxic degradants should involve interrogation of the parent structure both *in cerebro* and *in silico* for the presence of alerting structures as well as for chemically labile structures that can lead to either (1) preservation of an alerting structure or (2) creation of an alerting structure through one or more of the reactions shown in Tables 2−6. If this initial assessment does not reveal the potential for a structural alert in each of the theoretical degradants, further consideration should be given to the less commonly observed structural alerts shown in Table 1. In addition, the substructure search function in the D3 database allows for detailed structural searches and should be a helpful tool as well when searching for information about structural features in drugs that can lead to alerting structures in degradants.

As was noted earlier, the purpose of this manuscript was to understand and categorize potential degradation pathways that lead to common structural alerts for genotoxicity. It is important to emphasize that the suggested use of tools such as the Pharma D3 database to evaluate the potential for the presence of genotoxic alerts in drug degradants is really just the first step in the analysis towards assessing if an alerting structure is actually of real concern. It is critical to highlight that additional steps typically employed when evaluating the potential of impurities to be genotoxic, such as refined SAR analysis, Ames testing and literature reference work, often discount the initial concern for genotoxicity. For example, the most common structural alerts unique for degradants we found in the D3 database were aldehydes, α, β-unsaturated carbonyls, and primary aromatic amines. Both aldehydes and α, β-unsaturated carbonyls are often Ames-negative when tested using standard

Table 6. **Reactions leading to the production of an alerting structure: polyaromatic hydrocarbons**

Functional group in parent leading to degradation	**Mechanism** (in parenthesis are listed the names of the corresponding drugs in the Pharma D3 database exhibiting this degradation pathway)
Aromatic chloride in chloro-substituted diphenylamine 	Photodehalogenation reaction, leading to cyclization (Diclofenac, Meclofenamic acid) 

strains; as such, they are common structures and found frequently both in food additives and pharmaceuticals.[12−16] Another frequently found positive alert for genotoxicity in pharmaceuticals and corresponding drug degradants, aromatic amines, are also oftentimes Ames negative and their specific toxicity has been found to be highly dependent on their steric conformation.[17−19] As an example, 4-aminophenol which is mentioned in section 2 (see above) as a degradation product of acetaminophen, is found to be negative when tested in an Ames assay.[20,21]

In summary, the actual numbers for alerting structures mentioned in this manuscript are expected to overestimate the actual numbers of true genotoxic degradants.

5. Conclusions

Software programs such as ToxTree have become important tools in the evaluation of chemical structures for the presence of genotoxic structural alerts. This article has highlighted how some of the currently available information for evaluating alerting structures found in drug degradants can be used towards classifying structural elements in parent molecules that have the potential to lead to alerting structures during degradation. Continued interdisciplinary collaboration between toxicologists, analytical chemists, process chemists, pharmaceutical scientists and software experts will be essential for the continued expansion of knowledge about details of drug degradation and its implication for toxicity.

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