On Structural Alerts for Reactive Metabolites in the Recent Literature

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Having followed the scientific literature on reactive metabolites (RM) for almost twenty years, I have noticed a shift in how and what researchers publish. Formation of RMs from drugs is important because it is the main cause of idiosyncratic drug induced liver injury (iDILI), which has terminated many drugs in clinical development, latest the GLP-1 receptor agonist **danuglipron** from Pfizer [1], and caused withdrawals from the market (Wiki, not all due to iDILI, https://en.wikipedia.org/wiki/Category:Withdrawn_drugs) [2]. In addition, many licensed drugs cannot be used optimally because hepatotoxicity restricts their use.

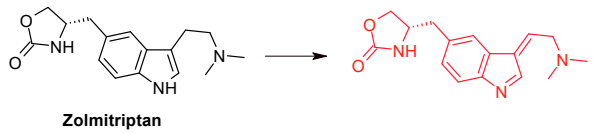
In the approximate period 1990 to 2010 drug companies showed a great interest in ways to minimize the introduction of clinical candidates with risks for causing iDILI. This was certainly spurred by several failures of recently launched drugs and clinical candidates in development, for example lumiracoxib, remoxipride, and many more. Chemical aspects of this strategy rolled high and biochemical testing and learning about structural alerts and mechanisms of bioactivation came very much into focus with researchers at the major drug companies showing great interest in contributing to this development [3]. Companies have tried many approaches but the simplest and most successful has been to just avoid certain substructures recognized as structural alerts [4] a development we have contributed to by developing the expert tool SpotRM that can readily identify > 95% of all known alerts [5,6].

After it has proven not so easy to routinely predict and collect what structures are riskier than others [7], the trend seems to have turned toward gaining more from the rapid development in biology and work with more advanced in vitro assays to identify early problems with important structures [8,9]. I leave to others to comment on this and will instead share with you a few observations made regarding what is published and from whom/where the research originates.

Mechanisms

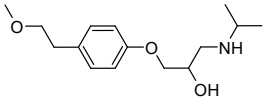
While each year several reports on new findings in the lead optimization process are published, there are few reports on novel surprising mechanisms of RM formation that would have been hard to foresee. Instead, well-known principles are reported to be active in known drugs, for example in **zolmitriptan** (3-alkylindol) [10], **ibudilast** (epoxide on heterocycle) [11], **metoprolol** (quinones) [12], and **donepezil**

(methide formation) [13]. It is interesting to note that all these examples have been published by Chinese authors, who seem to have taken over this niche of chemical toxicology.

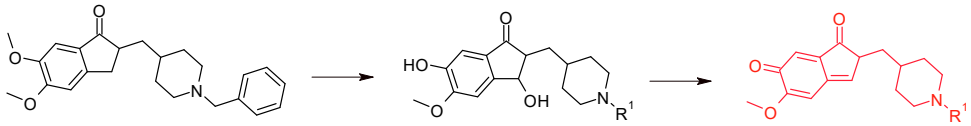


Zolmitriptan

Ibudilast

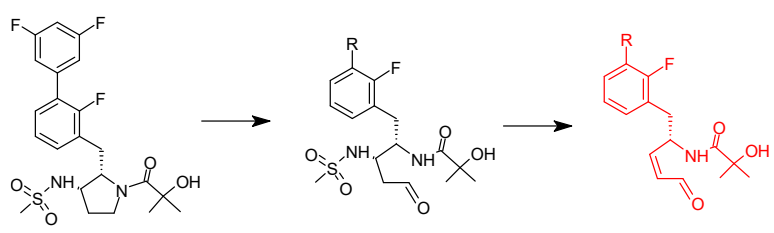


Metoprolol

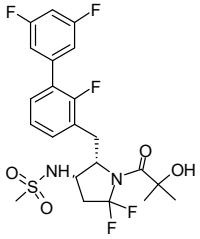


Donepezil

Mechanisms that have been reported in only a few cases over-all continue to be sporadic: one recent example is bioactivation of the orexin receptor antagonist **TAK-994** that caused hepatotoxicity leading to termination of its development [14]. Mechanism of bioactivation was not addressed but probably goes via oxidation alpha to N releasing an aldehyde that in turn eliminates a sulfonamido group eventually giving rise to an ∝,β-unsaturated aldehyde. By introducing fluor into an analogue (**TAK-861**) this bioactivation was elegantly abrogated. This is a rare type of bioactivation that has only been reported a couple of times during the last two decades.

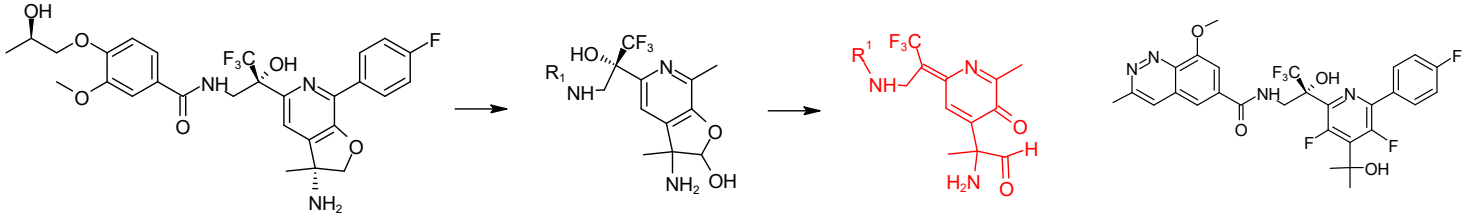


TAK-994



TAK-861

Quinone methide formation is a well-known route into the RM marsh (*cf.* **donepezil** above) that occasionally can be hard to anticipate [15]. Even experienced chemists can fall into this trap set by nature: during work on non-nucleoside RSV polymerase inhibitors, workers at Janssen found that the 6-aza-benzofuran below gave unacceptable amounts of RMs [16]. A mechanism was not suggested but we believe that the one drawn below is a reasonable supposition.



The Janssen chemists resolved the issue by abandoning the ether substructure and replacing it with the distinctly different structure **JNJ-8003** that was chosen as a clinical candidate.

Who publishes on RM mechanisms?

An interesting trend regarding who publishes on RM mechanisms can been found by analyzing occurrence of certain key words on the internet. The search expression "reactive metabolites" OR "reactive metabolite" tried on Google Scholar results in about 1300 hits for year 2010 and about 2400 for 2024. This increase, I think, might simply reflect more awareness of risks from herbal medicines (especially in Asia) but could also be augmented by Google having become very good at picking up spurious occurrences of various key words.

More relevant to the core of chemical toxicology is what is being published on avoidance of drug candidates with potential RM problems. Here, searches using the mentioned key words over all the ACS portfolio of journals revealed that the mentioned search expression has hardly increased in volume: all-in-all occurrence in 2783 references till April 22, 2025. Year **2024** 131; **2023** 100; **2021** 98; **2018** 95; **2014** 96; **2010** 108, that means a rather constant figure over many years. Regarding origin of publications one can note that in 2024 43% of the citations have "China" as cooccurrence while in 2014 the corresponding figure was only 18% (with a clear trend of increase over the following years).

Why are so many poor structures still published?

With the favorable development regarding knowledge of which substructures of drug molecules will be bioactivated to reactive species, one may raise an eyebrow over what is being published in the current medicinal chemistry literature; also commented on here [7]. For example, there one can see thioamides and thioureas being promoted as good starting points for optimization despite their well-known toxicity. Furthermore, the chemical group comprising hydrazides is represented with very few current drugs, for good reasons. Yet, a non-negligible share of articles in the *Journal of Medicinal Chemistry* is about such compounds as potential new drugs: in the period 2001-2005 there were 40 articles, in the period 2011-2015 there were 54, and 2020-2024 there were 88. That might be considered a little surprising. It is notable that these articles, as one could have guessed, did not originate from any of the larger pharma companies.

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