Liver Toxicity: Structural Knowledge May Help Avoiding Rare Drug Adverse Events

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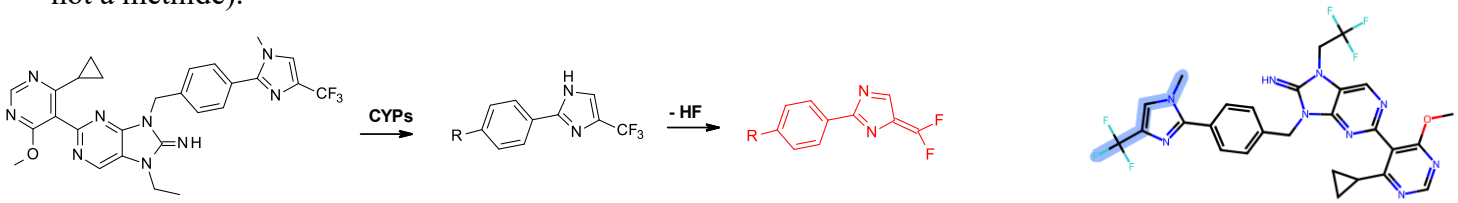
Many obstacles have to be overcome before a new drug can be launched but the challenges do not stop there. The approved drug being used in many more patients than in the clinical trials, rare adverse effects (AE) are more likely to show up. Especially problematic is idiosyncratic drug induced liver injury (iDILI), which has caused licensed drugs to be withdrawn or more often be relabeled. Since ~2010 regulators and companies have increasingly used label changes, boxed warnings, restricted indications, intensified monitoring requirements, or market withdrawal in specific countries only rather than global removals.

There is a large body on iDILI in the biomedical literature with a focus on diagnosis and identification of the culprit drugs. Also, there are discussions of the mechanisms, where "mechanism" is being limited to (the phenotypic) biochemical, physiological, and anatomical signs of liver injuries and how understanding of these can be utilized in new (in vitro) assays for screening of new test compounds. However, when it comes to avoid making problematic test compounds, an understanding of structural features is a prerequisite for guiding drug design. Since a few decades it has been recognized that formation of haptens from problem drugs is most often the cause of iDILI.[1] Here, formation of reactive metabolites (RM) has been shown to be the most common but also a very unpredictable source of reactive molecules while intrinsically reactive drugs, such as beta-lactams, are behind the rest of the neoantigens derived from drugs.

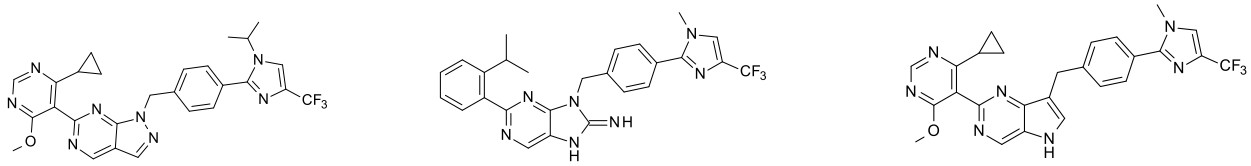
Since knowledge of how chemical compounds can be bioactivated to RMs has accumulated during a few decades, the design of much safer test compounds has become a realistic goal for medicinal chemists in the drug industry. This is certainly reflected in the statistics as hinted in the introduction. During the last ten years no major withdrawal of a drug due to liver toxicity has occurred but quite a few drugs have been targets of scrutiny by authorities and companies themselves. Even more significant, many investigational drugs or development programs were stopped, placed on hold, or prematurely terminated in clinical development because of liver toxicity. Since there are lessons to be learned from a drug design view, I will discuss some of these cases from the aspect of potential RM formation.

In 2024, the USP1 inhibitor **TNG348** was stopped in an early-to-mid stage cancer trial due to liver toxicity (Reuters). No chemical details have been disclosed about what might have caused the problems. However, from an RM warning aspect, the structure presents at least one suspect feature namely the trifluoromethyl group on the imidazole: twenty years ago, it was described how simple 2- and 4-difluoromethylimidazoles decompose within three hours at neutral pH in water via reactive imine methides.[2] This type of reaction applied to the current drug would lead to the following scheme for formation of an RM (some chemists would call this structure a diazafulvene not a methide).



TNG348 **Identified SA**

The application SpotRM (at spotrm.com) readily identifies this structural alert (SA) as the only one in this drug structure (above right).

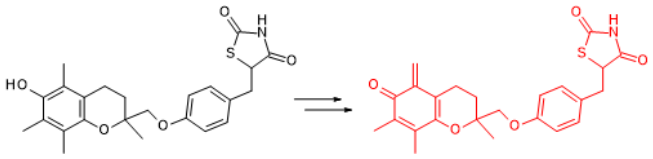


KSQ-4279 **I-138** **TNG-6132**

Now, there is precedence to the tried **TNG348** since it is a close analog of one of the first potent USP1 inhibitors, **I-138** (Forma Therapeutics around 2016), which for unknown reasons never entered clinical trials. Another USP1 inhibitor, **KSQ-4279** (**RO7623066**), is a close analog of **TNG348**, also having a 3-trifluoromethylimidazole (company link). This analog has passed Phase 1 clinical trials and still seems to be tried in patients (Aug. 2025).[3] If true that it would turn out to be less hepatotoxic than **TNG348**, one might speculate that a lower rate of dealkylation of the isopropyl group compared with a methyl could mitigate against RM formation from the imidazole group.

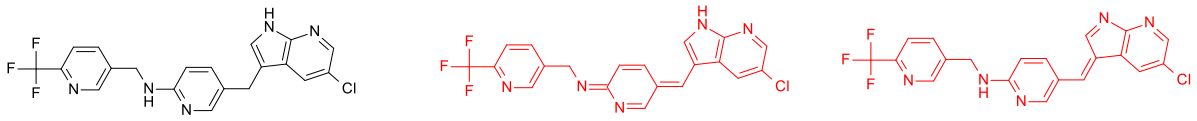
Last April, Tango Therapeutics published another USP1 inhibitor, **TNG-6132**, that contains the same trifluoromethylimidazole group as in **TNG348**.[4] This report does not mention potential RM formation nor state of clinical trials.

In general terms, this type of methide formation is well-known in benzene compounds having a leaving group on a methyl group *ortho* or *para* to a phenolic hydroxy group.[5] One often referenced example is **troglitazone**, a withdrawn PPARγ agonist, which is metabolically oxidized on a methyl group forming a labile benzylic alcohol (below). Many more examples are discussed by us in a 2022 perspective,[6] and more recent examples are also given in our recent blog post ("*On Structural Alerts for Reactive Metabolites in the Recent Literature*").



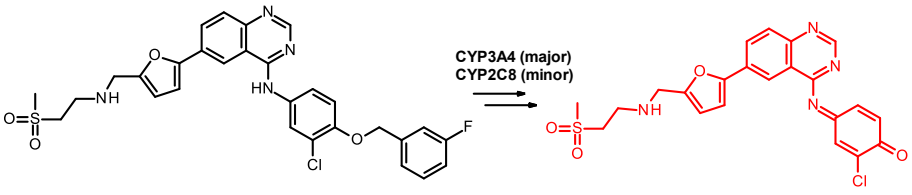
Troglitazone

Among licensed drugs that have risk of serious and potentially fatal liver injury, and with methides as RMs, it is highly relevant to mention the kinase inhibitor (KI) **pexidartinib** (Turalio®). This anticancer drug is licensed in the US with a black box warning but was not granted permission by EMA based on its poor cost-benefit ratio (link to motivation from 2020). Looking at the structure from an RM perspective, the two available ways to form methides (drawn below), and picked up by SpotRM, provide a strong indication of how toxicity might be induced. The conclusion is strengthened by recent information that these metabolic pathways are predominant.[7,8] From a technical perspective, according to the two publications, the metabolic pathway to the methide (or azafulvene) on the 7-azaindole seems to be dominating over the one over the aminopyridine part.



Pexidartinib **Two reactive metabolites**

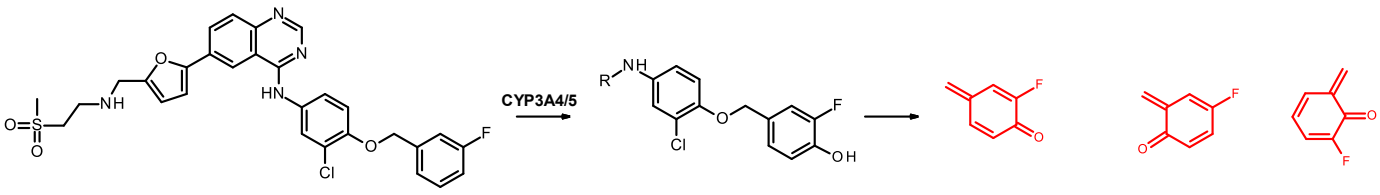
It is well-known that the drug **pexidartinib** is not alone among the KIs to have serious side-effects. On this theme, we have published an analysis of 25 KIs with known problems of hepatotoxicity and found that a majority of these have clear warning signs by presenting (well-known) SAs for RMs.[9] One of the oldest KIs mentioned there will be highlighted here because of new information on how its RMs are generated: **lapatinib** has been reported to generate a quinone imine by sequential debenzylation and oxidation reactions (below). The formation of the reactive quinone imine was tentatively supposed to explain most of the RM issues.



Lapatinib

However, in 2025 Genentech researchers revealed that the removed benzyl part might play a bigger role than previously tacitly assumed.[10] One might suppose that a benzaldehyde should be the only product from the oxidation on the benzylic carbon but this was proven wrong in the new findings. The authors tested alternative routes by extensive identification of metabolites, also using

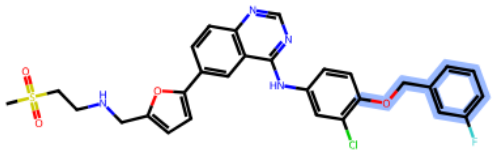
labeled compounds. They found that the formation of a phenol by *para* hydroxylation on the benzyl leads to a labile compound that spontaneously eliminates another phenol (as a phenoxide) to form a quinone methide as illustrated in the scheme below; *ortho* hydroxylations were also observed, leading to two different methides (below right).



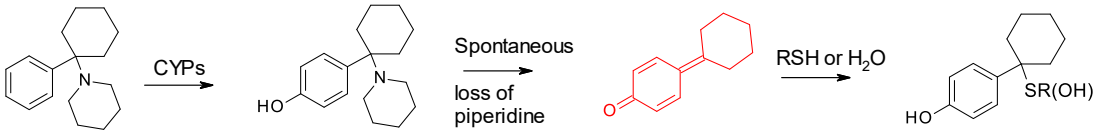
Lapatinib **Two other methides**

This kind of bioactivation has rarely been reported to play a role in RM generation but in SpotRM there is a SMARTS string that takes care of this possibility (illustrated below). In our SMARTS specification, the leaving group is limited to *phenoxides* while excluding *alkoxides*,

Warning detailed



which likely would be poor leaving groups. An amino group in that position has been shown to be a good leaving group: the example below is the first one published, in 2007, where the illicit drug **phencyclidine** was studied by Driscoll et al.[11]

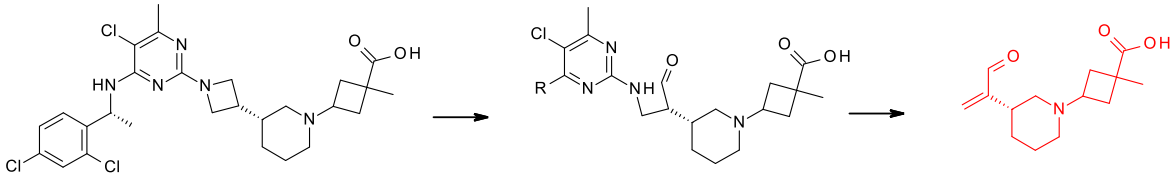


Phencyclidine

Other problem drugs having less apparent warning signs

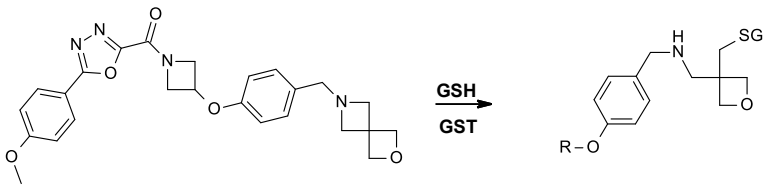
Although so much is known about structural features that increase risks for RM formation, experts may still have great difficulties spotting structural weaknesses in some hepatotoxic drugs, which in addition might have several minor liabilities that add up, even synergistically. In fact, in our experience a majority of the druglike compounds in the ChEMBL database contain at least one SA listed in SpotRM. To illustrate, it is not obvious by what bioactivation mechanism(s) the drug **zelnecirnon**, a new CCR4 receptor antagonist, forms RMs (which it probably does). It was discontinued in clinical trials in 2024 due to liver injury in one patient and was then stopped completely (link to company). One may not find it immediately obvious where the weakness(es) of the structure lies but it should not be far-fetched to think that the azetidine ring might cause problems, for example according to the scheme below. We and others[12] have commented on risks

with this strained ring and it is a SA in SpotRM; colleagues in the industry have also expressed concern. For example, a few years ago AZ researchers found that an azetidine can be ring opened with a thiol as nucleophile, specifically in a reaction with glutathione, a rection that yet requires catalysis by glutathione S-transferase (scheme containing **AZD1979** below).[13]



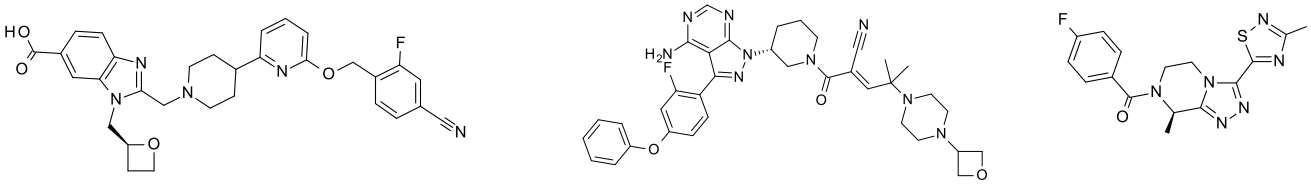
Zelnecirnon **Labile aldehyde?**

Regarding this unexpected reaction of **AZD1979**, one might note that the leaving group is an aliphatic amine, which is probably superior to an aromatic amine, such as in **zelnecirnon**?



AZD1979 **M12**

In April 2025 Pfizer terminated development of **danuglipron**, a GLP-1 agonist, after a case of potential drug-induced liver injury and after reviewing clinical data and regulatory feedback. No details of its reported metabolism have mentioned involvement of the oxetane ring but on the other hand one may note that the only approved drug (except **paclitaxel** and analogs) containing that strained ring is recently approved **rilzabrutinib**. This is an orphan drug for patients with persistent or chronic immune thrombocytopenia, which has severe DILI warnings (and showing two flags in SpotRM). The uncertain situation with oxetanes in drugs persists despite many efforts to apply them in design of new test compounds (*cf.* **AZD1979** above!). We have not yet included the oxetane ring as an alert in SpotRM but we have heard that some companies consider inclusion of it in their alerts.



Danuglipron **Rilzabrutinib** **Fezolinetant**

Finally, last December FDA issued a warning about rare occurrence of serious liver injury with use of the NK1 receptor antagonist **fezolinetant** (used for the treatment of hot flashes due to menopause). One tentative hypothesis might be that the alcohol formed on the methyl group,

which is a known metabolite (**ES259564** in the FDA assessment report), may form a reactive sulfate. This is the only alert found by SpotRM ("5m-Hetcycl w alkyl").

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