On Quinone Methides, Azafulvenes and Liver Toxicity

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We know that reactive metabolites (RM) come in many different forms and the current knowledge base is large. Yet it is not often that we can claim sufficient knowledge to say that a chosen drug structure will *not* cause RM problems. Part of the problem is probably that we could use knowledge more efficiently. To honor this message, I will highlight a subclass of RMs, **quinone methides** (QM), which I think has been treated somewhat stepmotherly in comparison with other bioactivation routes where arenes are involved. For example, the other "quinoids" (quinones, quinone imines, and quinone diimines) have received considerably more attention in the literature. An additional factor increasing the reporting on the other quinoids is their environmental impact stemming from natural products and manmade THB, a preservation agent. The imbalance can be roughly appreciated by the following Google Scholar searches, Nov25:

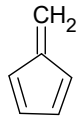
"quinone imine" OR "quinones" AND "drug metabolism", ~4100 hits since 2016.
"quinone methide" AND "drug metabolism", ~480 hits since 2016.

In a perspective paper in JMC 2022 we tried to straighten up the imbalance by focusing on the QMs and in the same report include the related *azafulvenes*, more below.

We know for sure that the reactivities of quinones vary a lot depending on the substituents and nucleophiles and this should mean something for their varying degree of toxicity. The problem is the usual one: we do not have a complete answer. However, based on their overall molecular makeup, that is a polar zwitterionic structure, QMs are generally more reactive than other quinoids, see e.g. a 2011 review and reactivity is also referred to in the key roles of QMs during the oxidative biotransformations of a vast array of catecholamine derivatives. These dipolar contributions in QMs are illustrated in Example **2** below.

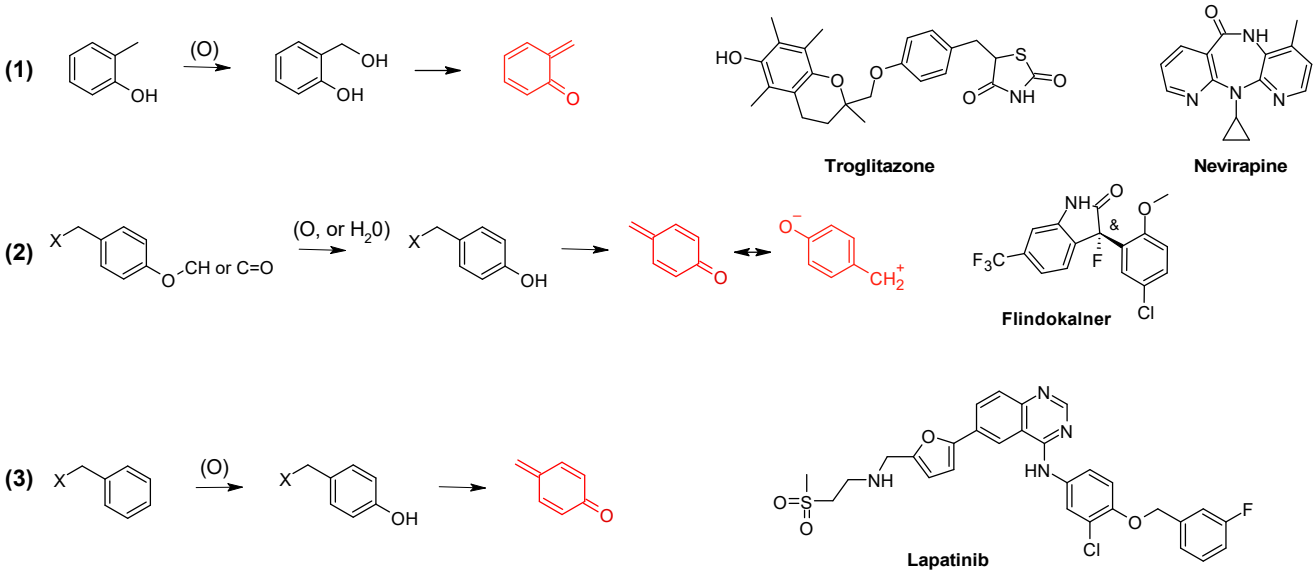
There are a few principal ways along which QMs on benzene (and analogous 6-membered heterocycles) can be metabolically generated; these are summarized in an ultra-short mode below, routes **1**-**3**. They have all been indicated in real drugs, as shown by the examples. For obvious reasons, the routes are available for *ortho* as well as *para* substituted precursors but not for *meta*. In addition, there is an isomerization path from *ortho* benzoquinones (not shown), which lacks practical importance and therefore is rarely mentioned.

What about analogous reactive structures based on five-membered heterocycles (FMH), so common in drug structures? The parent structure is a hydrocarbon, the benzene isomer fulvene, which is of minor interest from a drug perspective since a precursor of fulvene itself can hardly be



Fulvene

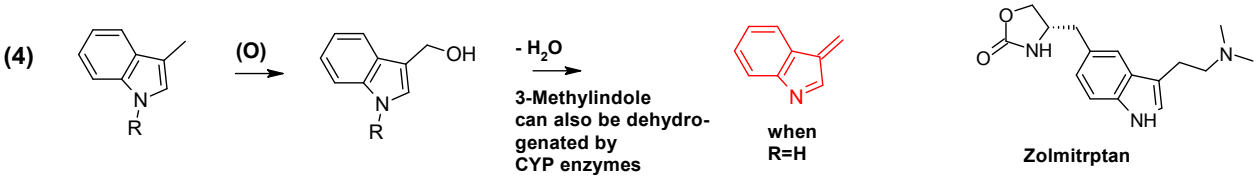
found among current drug structures. Instead, the most common one in the RM arena is the fused heterocycle 3-methyleneindole (Example **4**), reflecting the great popularity of indoles in medicinal chemistry (a foregone period?). Formation of this reactive species is illustrated in Example **4** and many more examples are listed in the app SpotRM (free access for academics!). In the following I will refer to

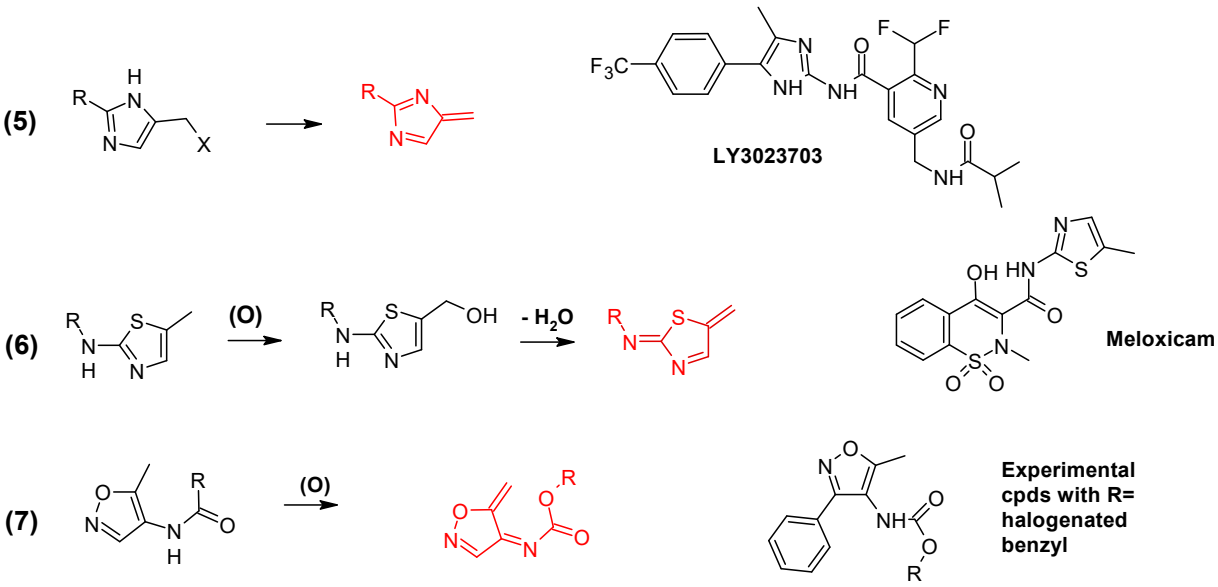


heterocyclic analogs of fulvene as aza-, diazafulvene etc. dependent on the hetero atoms. Much more variation of reactive structures is to be expected here compared with the mentioned QMs because of the many possible variations among FMHs, including fused rings with other aromatics.

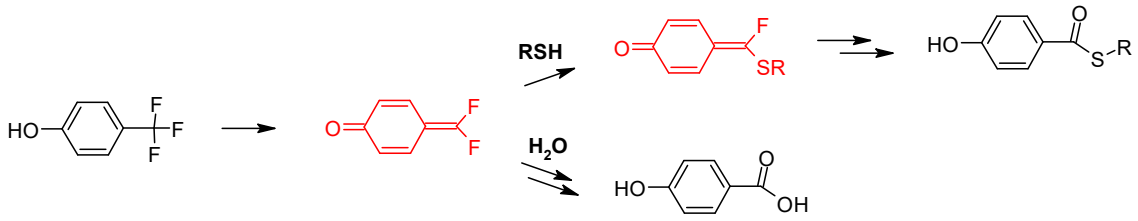
Ways of forming fulvenes containing hetero atoms are very much analogous to the six-membered ring QMs and follow the same principal paths, often with an initiating oxidation to an alcohol. However, the reactive products, the RMs in a drug context, have rarely been clearly indicated and identified as in the QM cases. This, in my view, is bothersome since there are quite a few cases that could have been investigated and the culprit fully and readily identified.

Now, this introduction could be a start of a lengthy blog post but not so. Readers who want to dig deeper into the subject are referred to the JMC22 paper and to the app SpotRM. Here, I will just go into detail regarding certain structures having a *fluorinated methyl* group on a heteroarene since they represent an easily avoidable danger that also has some current relevance.





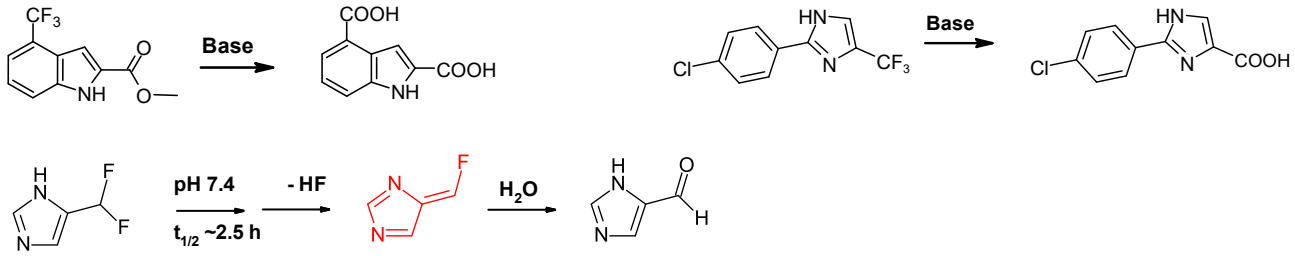
Benzylic fluorides as RM precursors. This special aspect of QM formation is mechanistically well understood and valid for formation of both QMs and azafulvenes: it is known since 1947 that fluorinated methyl groups on phenol result in unstable compounds that decompose with loss of fluorine. As mentioned, this only occurs with *ortho* and *para* substituted phenols and the mechanism is straightforward, exemplified here below with **4-trifluoromethylphenol**: elimination of HF (E1cB mechanism) occurs readily from the phenolate anion creating a very reactive methide, which in turn reacts with any nucleophile present; water gives complete hydrolysis while thiols give (relatively stable) thioesters, and amines result in amides. The last-mentioned reaction is also useful in organic synthesis of tertiary amides. In vivo, the risk of forming new antigens from proteins is obvious.



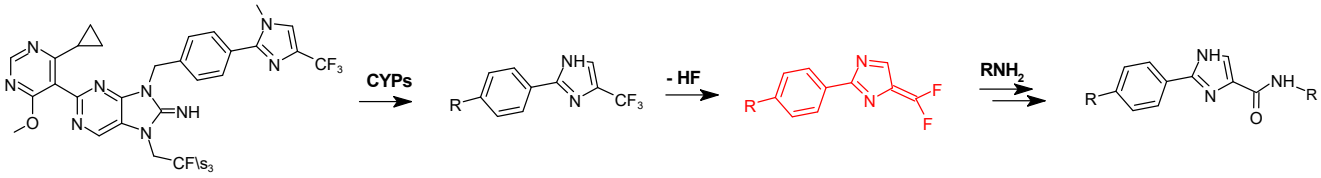
Drugs that can release a 2- or 4-fluoroalkyl-substituted phenol are rare but do exist, for example the well-known antidepressant **fluoxetine**. Release of 4-triluoroalkylphenol, which is a reported pathway, is shown below.

Fluoxetine

Similarly, HF can be eliminated from an alkyl group on a FMH. This is known since at least 1957 when Bornstein et al. discovered that **5- and 7-trifluoromethylindoles** are labile under basic conditions as shown below for methyl indole-2-carboxylate. The same discovery was made by Baldwin et al. in 1975 regarding **4-trifluoromethylimidazoles**. In complete analogy, **4-difluoromethylimidazole** in water at neutral pH is unstable (JFC06).



These observations as a background lead us to present times: in a previous blog post I mentioned the USP1 inhibitor **TNG348**, which in 2024 was stopped in an early-to-mid stage cancer trial due to liver toxicity (Reuters). No details have been disclosed about what might have caused the problems. However, from the facts presented above it seems probable that formation of a very reactive difluoromethyleneimidazole (a fulvene) might have caused the problems. This could react with sulfhydryl or amino groups in proteins forming thioesters or amides as new antigens.



TNG348

A few other analogs that have an N-alkylated 4-trifluoromethylimidazole were also commented on in the same blog post. One had a N-isopropyl group instead of a methyl, which might have resulted in less N-dealkylation and thus less RM formation.

To summarize, the message in this post can be interpreted in a positive way: I suggest that the dangers in drug design regarding bioactivation of alkyl groups on aromatics can be handled by spotting certain readily recognizable patterns of substituents over aromatic rings (well suited for rule-based learning). This drug design problem can, for example, be compared with that of the much harder one to identify which epoxidations of aromatic systems can be predicted to cause problems (based on often scarce medical/biological data, that is). What is lacking regarding certain hetero-fulvenes is knowledge about to what degree they can be formed energy-wise, and of course, there may be other blind spots as well.