On Elimination Reactions and Reactive Metabolites. Part II

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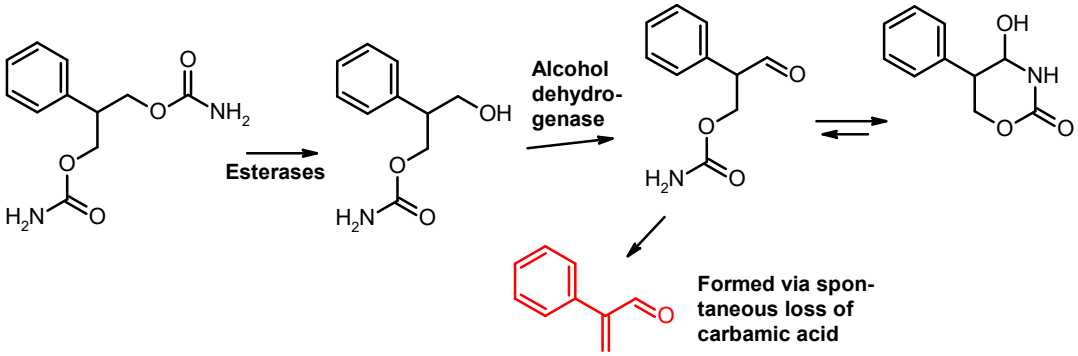
This post is the second on how different groups can behave as leaving groups (LG) in elimination reactions. Here, focus is on examples of how an initial oxidation leads to formation of *conjugated keto compounds*. Part I was about formation of *quinone methides* initiated by hydroxylation on aromatics or release of a phenol by O-dealkylation.

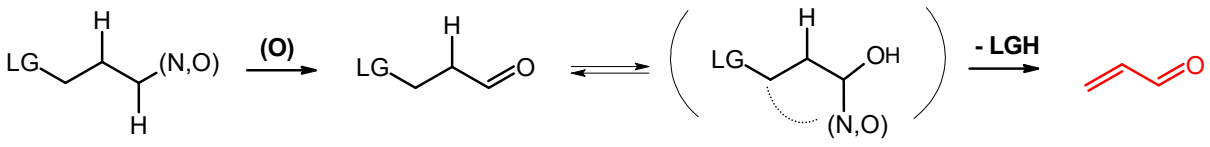
LGs in formation of conjugated keto RMs. While not being extremely well represented in the group of verified reactive metabolites (RMs), ∝,β-unsaturated carbonyl compounds are certainly not welcomed as metabolites. Some representatives have a particularly bad reputation: alpha-phenyl acrylaldehyde (2-phenylpropenal, atropaldehyde) from the high-dose antiepileptic **felbamate** gives rise to serious side-effects. The drug has a black box warning for aplastic anemia and risk of hepatic failure. In this case the unsaturated aldehyde is generated by a 1,2-elimination of *carbamic acid*, an unstable compound, which serves as an excellent LG.[1] This combined with the formation of a benzene-conjugated double bond provides a particularly low threshold for the elimination.

Scheme 1



While the initiation of RM formation from felbamate is rather unique, ∝,β-unsaturated aldehydes (and ketones) are more often formed as RMs according to the general Scheme 2 starting with an alpha-carbon oxidation. Only cyclic amines, particularly pyrrolidines and at least one piperidine are represented in the literature. Here, the oxidation creates a cyclic hemiaminal in

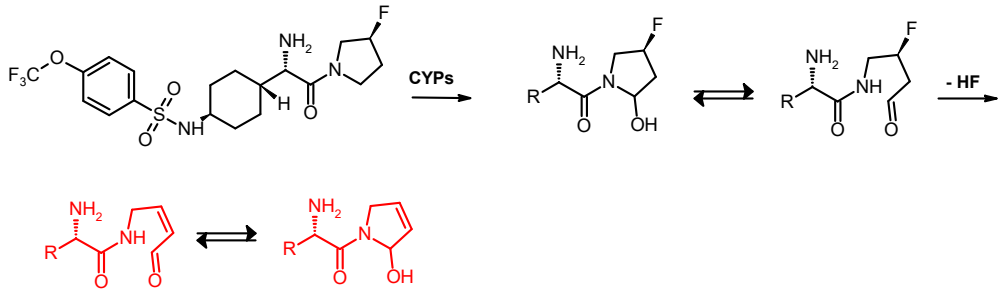
Scheme 2



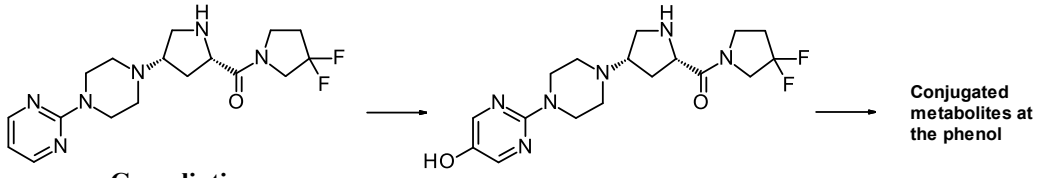
equilibrium with the keto group. In an ensuing step, the LG in the 3-position undergoes elimination (relevant for non-cyclic structures, too). A few examples are briefly mentioned in reviews on the general theme "Alicyclic Amine Bioactivation to Reactive Iminium Species";[2–4] focus is throughout on the roles of iminium species as potential reactive metabolites.

Regarding pyrrolidines, the above elimination mechanism has been shown to be active in, e.g. the **Merck DPP-4 inhibitor** in Scheme 3 that bound irreversibly to rat liver microsomes in a NADPH dependent manner.[5] A *fluoride anion* serves here as an effective LG. The analog **gosogliptin** in Scheme 4 would be thought of as a much worse offender as an RM precursor since it could create a more reactive aldehyde. It certainly can but drug properties and metabolism come in the way.[6] As seen in the same scheme, the rather hydrophilic gosogliptin is rescued by forming an even more hydrophilic metabolite and the problem is solved.

Scheme 3



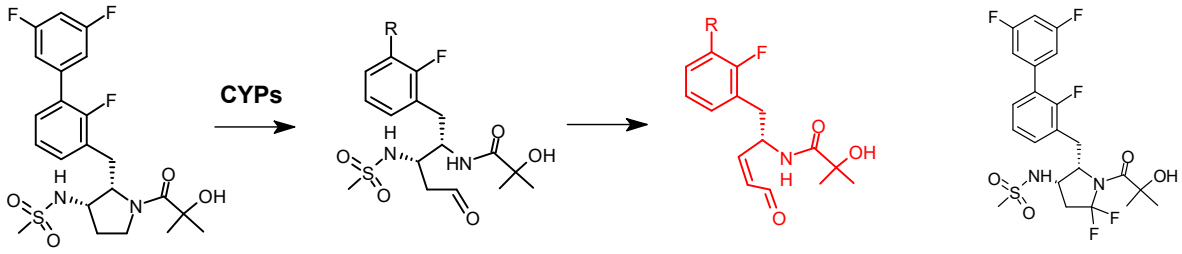
Scheme 4



Gosogliptin

A follow-up question to the presented case may arise: which other groups could act as LGs in this situation and therefore would not be "safe" to include when an ∝,β-unsaturated keto compound is at risk of being formed? Another example might take us one step further to help answer the question: it seems likely that the recently reported liver toxicity problems of the orexin receptor

Scheme 5



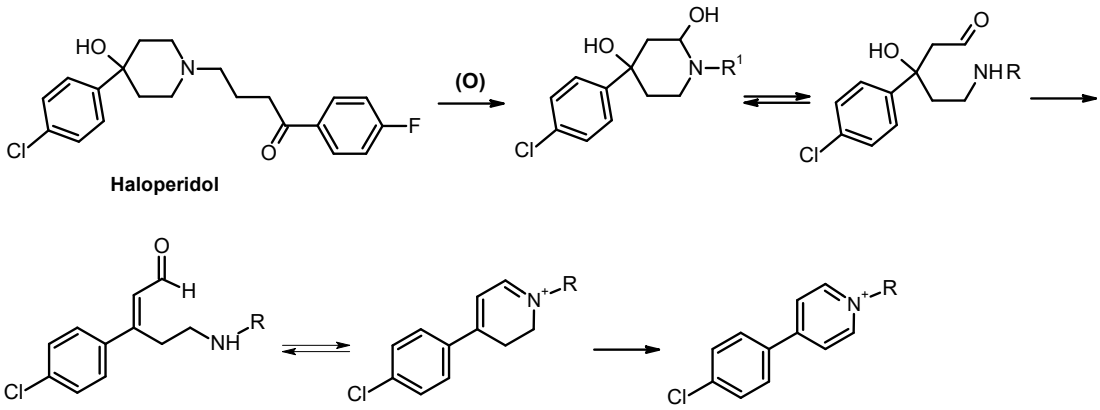
TAK-994 **TAK-861**

antagonist **TAK-994** are caused by the reactions in Scheme 5.[7] These activation steps are the same as in Scheme 3, just involving the different LG, *methanesulfonamide*. The mechanism was more

or less corroborated through synthesis and testing of the fluorinated analog **TAK-861**, which didn't exhibit these problems.

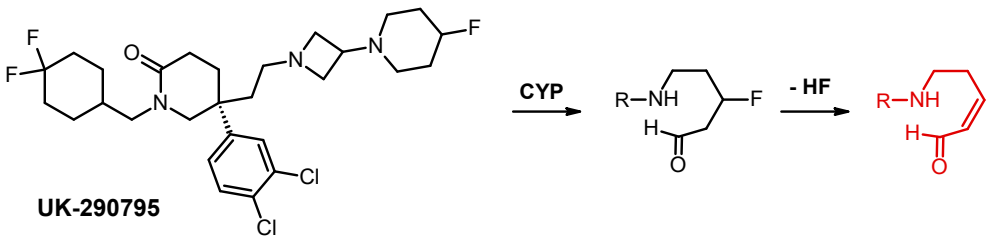
The sequence described has also been observed in piperidine where the most (only?) studied drug is **haloperidol** (HP) although the reports do not seem to provide evidence of formation of trapped products from a formed RM.[8] It has been known since the 1990-ies that HP can generate a pyridinium metabolite (Scheme 6), which has been implicated in causing brain injuries analogously to the neurotoxin MPP+. Somewhere along the route, the hydroxy group of HP is eliminated, but is a "true" RM really formed? What is apparently lacking in the scheme is proof of adduct formation (with GSH) from an RM, and the required conjugated aldehyde does not seem to have been identified and reported. Also, I have not found that aldehyde trapping with semicarbazide has been reported and studies on covalent protein binding seem to be lacking too. However, the formed hemiaminal shown in Scheme 6 has been trapped with cyanide, generating two diastereomers.[9]

Scheme 6



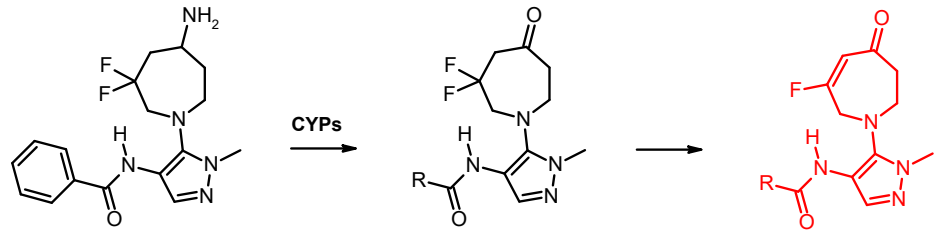
A piperidine with a likely larger potential than HP for forming a reactive ∝,β-unsaturated keto compound is the NK2 antagonist **UK-290795**, which was terminated in development, allegedly in clinical trials around 25 years ago. Elimination of HF could here provide a more reactive ∝,β-unsaturated aldehyde than in the HP case.

Scheme 7



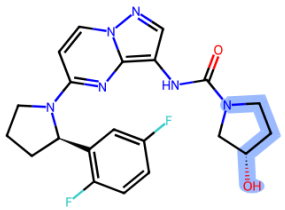
Another cyclic amine that has the correct alignment to form a reactive keto compound is the **difluorinated azepane** in Scheme 8, a PIM kinase inhibitor.[10] It was shown to give rise to time-dependent inhibition (TDI/MBI) of CYPs, likely via formation of a reactive 3-fluoro-enone as corroborated by studies on model compounds. Since the bioactivation is dependent on initial formation of a ketone, a methyl was successfully inserted to block the alpha oxidation.

Scheme 8

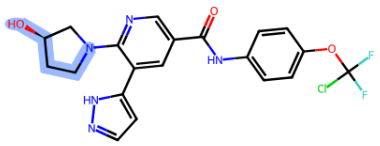


PIM kinase inhibitor

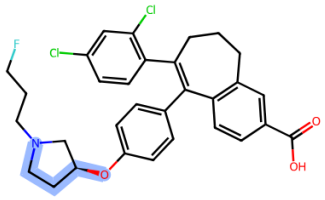
Other hypotheses. Just a few examples were mentioned above as verifications of a valid Scheme 2 for causing RM issues in drugs, in fact a structural alert model. However, when continuing the discussion of cyclic amines, one can note that a few other pyrrolidines exhibit an interesting pattern that might induce formation of unsaturated aldehydes. The two pyrrolidines mentioned were reported to form ∝,β-unsaturated keto compounds by loss of two different LGs, and HP loses water initiated by amine oxidation (but with no verified RM consequences). The TKI drugs **larotrectinib** and **asciminib** both feature a 3-hydroxypyrrolidine, an alert from SpotRM is marked on their structures below. It might be interesting to find out to what extent alpha-N oxidation of these pyrrolidines would lead to elimination of water. Potential RM formation, which probably is not extensive, could certainly be investigated experimentally.



Larotrectinib



Asciminib

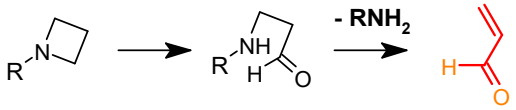


Amcenestrant

Another pyrrolidine, **amcenestrant** was in phase 3 clinical development with Sanofi but was stopped in 2022 after showing non-satisfactory results as a selective estrogen receptor degrader. It also showed certain liver injuries, which, however, were dog specific. As shown on its structure above, the phenoxy-substituted pyrrolidine might be relevant for studies of RM formation, which would also provide generally useful information. Phenoxide (phenolate) has been shown to be an efficient LG in methide-forming bioactivation of lapatinib[11] (also in the previous blog post); see also other hypotheses of phenoxide as LG below.

In the above examples of cyclic amines from the literature, **azetidine** is lacking despite having an extra potential to generate ∝,β-unsaturated keto compounds according to Scheme 9. Here, it seems clear that the properties of the RNH_2 as an LG combined with the influence from other groups on the ring will determine the outcome.

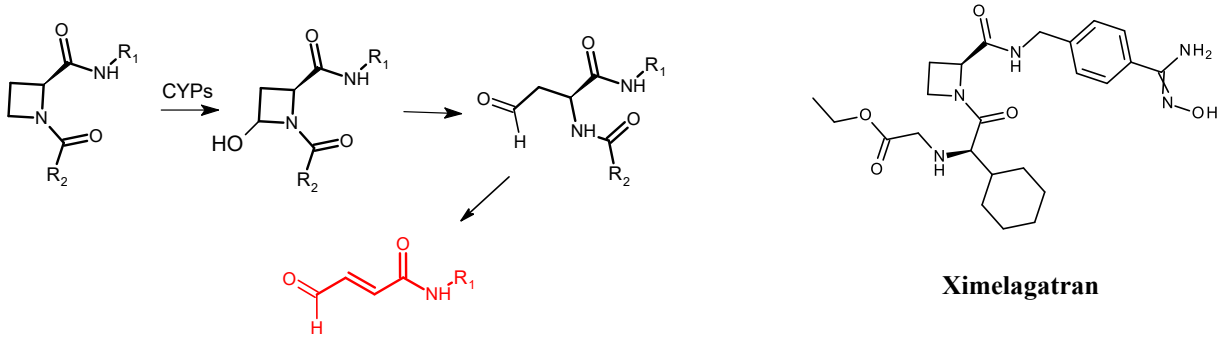
Scheme 9



Reported examples are hard to find but in 2024, the withdrawn drug **melagatran** (including its prodrug **ximelagatran**) was mentioned by me as a potential precursor of an ∝,β-unsaturated keto

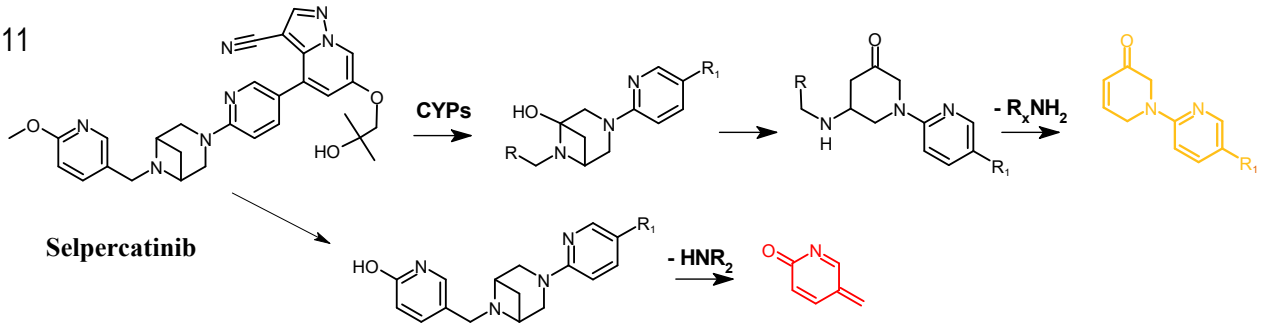
compound.[12] The hypothetical steps are shown in Scheme 10. The withdrawal of the approved drug due to DILI issues back in 2006-7 caused consternation at AstraZeneca but a plausible RM or chemical mechanism was never reported.[13] However, formation of an aldehyde RM as shown might be a partial explanation since it is driven by formation of a highly conjugated system, an amide of succinic semialdehyde.

Scheme 10



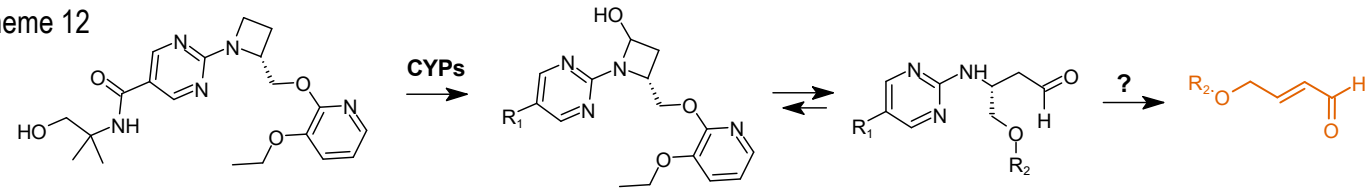
One could easily imagine that the azetidine **selpercatinib** might form an unsaturated ketone by being oxidized on an azetidine alpha-carbon and the formed ketone then eliminating the amino group (Scheme 11). But how problematic would the product, a substituted dihydropyridine-ketone be, here marked in yellow? A more likely explanation of, or contribution to the rather serious hepatotoxicity of this drug is illustrated in Scheme11 where a methide is easily formed by elimination of an azetidine group (this sequence was accidentally omitted from Part I of this two-part post where only one amine as an LG was highlighted).

Scheme 11



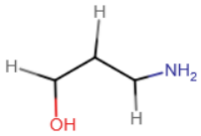
As mentioned in the introduction, iminium ions and keto compounds as potential RMs have been discussed and debated in the literature. The transformation in Scheme 12 is from a study of a Pfizer drug, an azetidine.[14] Here, the focus was on the aldehyde, which was identified as a semicarbazone and a cyclic thioaminal derived from breakdown of a GSH adduct. One might here imagine a loss of the pyrimidine-2-amine that would lead to an unsaturated, reactive aldehyde. This RM was not reported and the reason is most likely that the aromatic amine is not a sufficiently good LG (pK_a around 3.5 for the parent amine). In addition, unlike the melagatran case, there are no other substituents that could promote a 1,2-elimination.

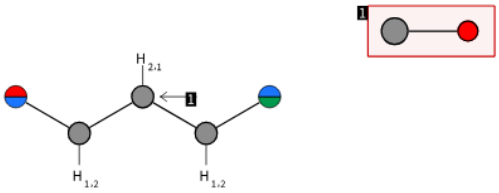
Scheme 12



Pfizer cpd

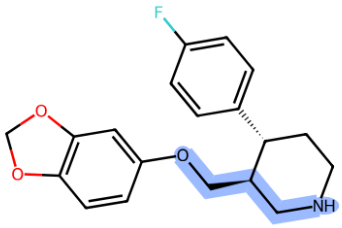
Many other drugs have a substructure that can be classified under the current Scheme 2 umbrella that also includes non-cyclic compounds. A structure search in DrugBank using the broad substructure below (no explicit H:s on N,O) and filtering on "Approved drugs" gives as many as 100 hits. This would of course not be a realistic structural alert for use in in-silico screening of planned test compounds (cf. Footnote) but for our purposes a closer look at a few example



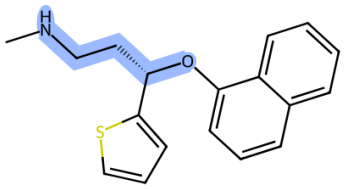


Substructure used to search Drugbank **SpotRM alert visualized with SMARTSplus**

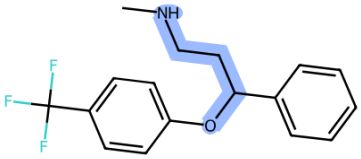
structures provides some unexpected insights. The following psychoactive drugs can be noted as hits in DrugBank: **atomoxetine**, **paroxetine**, **fluoxetine** and **duloxetine** all have structures that can be oxidized to a 3-phenoxy aldehyde; the alert used in SpotRM is marked below on three of the structures. Do these aldehydes, which should to some unknown extent be formed in vivo, eliminate phenols and give rise to unsaturated aldehydes, as outlined for atomoxetine in Scheme 13? This could certainly be investigated.



Paroxetine



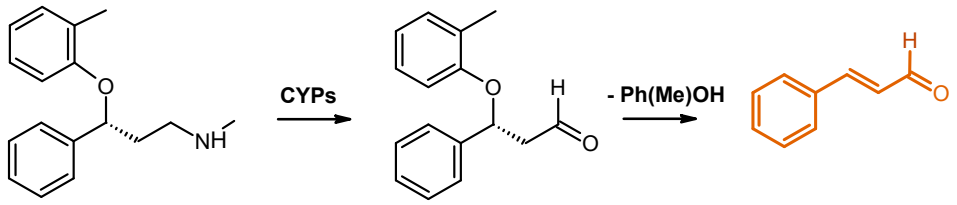
Duloxetine



Fluoxetine

Another question is whether these RMs, if formed, would make a decisive contribution to the adverse effects seen in the clinic? My guess is, probably not since known RM formation from metabolism of the aromatic parts should dominate. This of course does not mean that the extra RMs formed would have no negative effects (*cf.* Footnote) These drugs are all used at a low-intermediate dose and are not known to have serious side-effects, but yet there are signs that might

Scheme 13



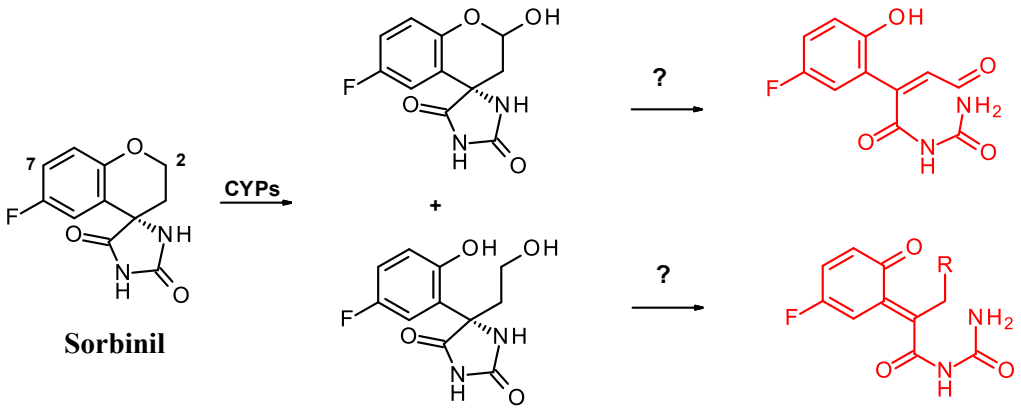
Atomoxetine **Labile aldehyde?**

be interpreted as being caused by RMs and RMs from the aromatics have also been identified. One may, e.g. note that atomoxetine has the warning "Postmarketing reports indicate that atomoxetine can cause severe liver injury. There is a publication on this, too.[15] The same is true for duloxetine.[16]

A more relevant question in the current context is how fast are the ∝,β-unsaturated aldehydes formed or put differently, how fast are the phenols eliminated (cf. amcenestrant above)? There is no answer in the literature but an indication may possibly come from the methide-forming reaction of lapatinib which seems to be fast,[11] (also mentioned in Part I)

Like alpha-carbon oxidation of an amino compound, analogous oxidation of H-C-O gives rise to a keto compound and an ∝,β-unsaturated keto compound may potentially be formed. This makes, e.g. the withdrawn drug **sorbinil** a potential RM precursor via the upper path of Scheme 15. Here, a new kind of LG would be involved. The lower path would form a methide, nicely connecting the two parts of the current comment (see Part I). Sorbinil was in failed clinical trials around 1985, with the patients suffering from serious hypersensitivity reactions; citation from SpotRM: "Riley *et al*. (BJCP1988) and Spielberg *et al*. (Ann1991) did experiments with microsomal metabolism and concluded that sorbinil is oxidatively metabolized to one or more potentially toxic intermediates, the nature of which have not been elucidated."

Scheme 15



Sorbinil

To summarize, comments here were made on metabolic routes to ∝,β-unsaturated keto compounds with strict focus on 1,2-elimination reactions. While not being recognized as a very dangerous kind of RM, it seems that their formation via this mechanism can be well anticipated.

FOOTNOTE

A basic conclusion about reactive metabolites (RM) is that minor formation of these will not be noticed in the clinic since the consequences are generally not noticeable. Also, approved drugs

have very rarely been investigated in detail regarding all metabolites formed, for obvious reasons. However, logically deduced from current knowledge of drug metabolism, most drugs should give rise to some degree of RM formation; one might just think of the unfortunate prevalence of benzene rings in current drugs, which will give rise to epoxides, and more. There should exist a "dark pool" of RMs as background noise not reaching a level that too negatively affects the clinical benefit-risk balance.

A master's thesis, not yet published in full, illuminates the dilemma: Leithner, H. "Investigation of Structural Alerts for Predicting Toxicity Linked to Reactive Metabolites", Wien, 2024. https://utheses.univie.ac.at/detail/72123/#. This is a result from a collaboration between Prof. J Kirchmair and Awametox AB, which developed SpotRM. Not very surprisingly, it was found that a large majority of the SMARTS used in SpotRM gave many hits in a subset of the ChEMBL database that features more druglike structures. The interpretation of this should be just that: a lot of RMs are formed all the time but mostly in so small amounts not to have consequences in the clinic. This should also be reflected in any attempt to list structural alerts for RM formation; the decision for database constructors between what to include and exclude is a fine balance. To try to help the overwhelmed drug designer on the receiving end we have included in SpotRM a danger scale of alerts ranging from 1-10.

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