Classification of Structural Alerts for Reactive Metabolites Based on Potential Danger

White paper

Awametox AB. E-mail: info@awametox.com

1. SAFER DRUGS CALL ATTENTION TO AVOIDANCE OF REACTIVE METABOLITES.

Elimination of all nontarget-related side-effects of a drug may not be a realistic goal, though aiming to avoid the worst ones is. This should be done at the earliest point in time possible, meaning at the drug design stage, i.e. before synthesis. The attention on safety at a very early stage includes many aspects but our focus here will be on how to avoid the risk of adverse drug reactions (ADR) caused by reactive metabolite (RM) formation.

In most therapeutic areas it is extremely important to avoid hepatotoxicity issues, in particular idiosyncratic drug induced liver injury (iDILI), when selecting a clinical candidate. Being forced to stop drugs in clinical trials or even worse, to withdraw an approved drug is highly negative from any aspect. It is generally recognized that the problems originate from covalent binding of drugs to proteins creating neoantigens that affect immune responses.[1,2] The pathway most often involves reactive metabolites but also direct reactivity of the kind that, for example is being built into beta-lactams and alkylating anticancer agents. An attempt to illustrate the events leading to an iADR is found in Fig. 1 (from Uetrecht et al.[3]) Here, our focus is on the very first steps in the formation of RMs by identifying signals to risky substructures.

The most direct approach to identify risks is recognition of structural elements that can generate RMs via enzymatic activity. Many such structural alerts for reactive metabolites, here SARMs, have been identified during the last 50+ years and have been applied to test compound deselection.[4,5] A web application that is strictly focused on identifying SARMs is SpotRM,[6,7] developed by Awametox AB. Its database holds a large collection of these alerts coded in SMARTS format. That does not currently include identification of directly reactive substructures since these should be easier to recognize. Other approaches to SARM detection focus on structure-toxicity by computational methods but these seem to have had less impact. One exception may be risk for quinone formation that is relatively easy to identify.[8]

Although seemingly a very simple concept, hands-on application of SARMs analysis involves several complications and uncertainties that still make assessment of the potential danger of a compound difficult. We will try to describe some of these in the following.

At the outset we know that 1) the dose and 2) the degree of metabolism to RMs are very important factors that determine the "body burden" of covalent binding.[9] However, at the stage of selecting a synthetic target it is not very meaningful to consider an estimated dose; instead one should focus on degree of conversion to RMs and the type of RM formed. The chemical nature of the chemical substructure, as the precursor of an RM, should be the determining factor for ease of metabolic activation. Regarding type of RM formed it is obvious that certain RMs are more reactive than other ones, for example a radical being more reactive than a benzoquinone (which in turn can have a great span of reactivity depending on substitution pattern). However, this is not to say that a more reactive RM is generally more dangerous. Differences between RMs should also extend into what kind of targets they react with, including type of nucleophile in the macromolecule.

These aspects (together with other common SAR aspects of drug design) might lead to the reasonable presumption that each RM precursor, i.e. SARM, should have its own danger profile, which might be captured by a severity score. Which proteins that are modified should depend on where in the body the RM is formed and as mentioned, its unique kind of reactivity. Attempts have been made to identify which proteins are hit by certain drugs (i.e. in most cases their RMs) but this seems to have been difficult to link to iADRs.[10]

Downstream to these considerations are the differing structures and biological activities of the so formed drug-protein

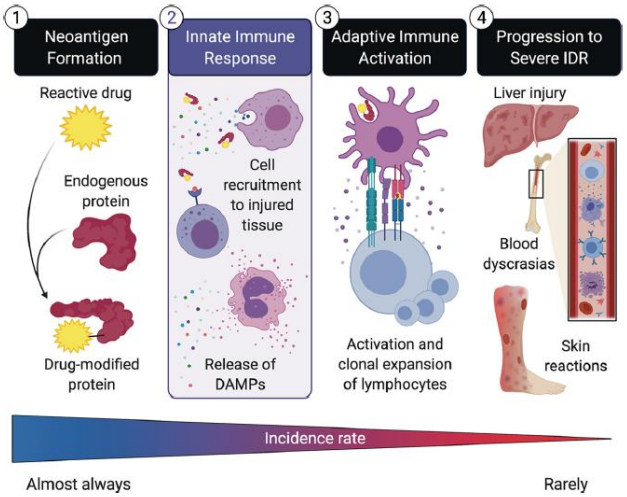

Figure 1. From Uetrecht *Curr. Res. Toxicol*. 2022, **35**, 1649

conjugation products (adducts). This is, however, an area where understanding is lacking, and it is probably correct to say that it has hardly started to emerge. It does not seem meaningful to say more than that the concept has high relevance to consequences of RM formation. One might,

for example, perceive that small drug molecules, like paracetamol, give fewer iADRs than larger ones because of smaller changes of protein structure in the product. Furthermore, the little knowledge that exists is limited to the directly reactive beta-lactam antibiotics.[11]

2. HOW TO DIFFERENTIATE THE DANGERS OF SARMs BASED ON THEIR CHEMISTRY?

Our main objective here is to discuss options for how the process of awareness/avoidance of RMs can be refined by better ranking of SARMs. Unfortunately, there is a major obstacle to this because of scarcity of background information. A proven association of a substructure with iADRs is relatively rare even when good hypotheses abound. It is probably correct to surmise that most clinically used drugs listed in SpotRM (250+) as Red or Yellow do not have a proven link to a validated chemical mechanism. In addition, in most cases the picture is blurred by the presence of more than one hypothetical SARM. The over-all problem of reaching a balanced decision to include/exclude a substructure as a SARM was discussed by Claesson based on an analysis of liabilities of recent kinase inhibitors.[7]

The pursuit of establishing a severity score of SARMs in SpotRM based on the above criteria is an experiment that might help selection of synthetic targets. However, we are fully aware that for a long time it will only be of marginal help.

Differences in substitution patterns of likely SARMS could possibly help in forming a basis for their classification but the crux is that the concept would require many sub-SARMs that reflect different substitution patterns, for example on a benzene ring. This is hardly a realistic starting point. Other alternative ways of looking at the ranking problem should take a broad view and build on all learning from the decades' long experience of various types of fundamentally different RMs. Here, we propose the following crude classification scheme based on three cornerstones.

- General experience from proven or likely SARMs
- Estimated reactivity/target preferences of the RM
- Degree/rate of conversion into RM

This allows us to form at least three groups A-C based on approximate ranking scores 1-10.

Group A, score 8-10. We know, for example, that aromatic amines have consistently proven to cause serious side-effects such as carcinogenicity and iDILI. This is valid for benzeneamines while heteroaromatic amines show a much more varied picture.[12] Hence it is reasonable to place benzeneamines in a "very red" category, score 9-10. Since various N-alkyl- and N-acyl-benzeneamines can be precursors of primary benzeneamines, these should be lumped together into Group A. Another structural feature that adds to the danger of certain benzeneamines is the potential for para-hydroxylation to form amino- or amido-phenols that are readily oxidized to reactive quinone imines.

Aromatic nitro compounds are reduced by multiple enzymes thus forming immunogenic nitroso compounds.[13] They are thus placed in Group A, too.

Other SARMs that should have a high severity score are the ones that give rise to methides of various kinds. This was the topic of a review by us, which is captured in an overall generalization in Figure 2, where formation of the major types of methides is generalized, including fulvene-like five-membered rings having an exocyclic methylene.[14] (For example, one alert originates in 3-methylindole, Figure 3). The name of the alert in SpotRM is "5m-N-heterocycles w 3-alkyl" and the SMARTS string will hit fused and non-fused rings. These heterocyclic substructures are found much less frequently in drugs than most benzene-containing SARMs but they are still alarmingly common. As to a typical methide based on benzene, the kinase inhibitor pazopanib forms a reactive benzylic alcohol that can generate a methide (Figure 4).

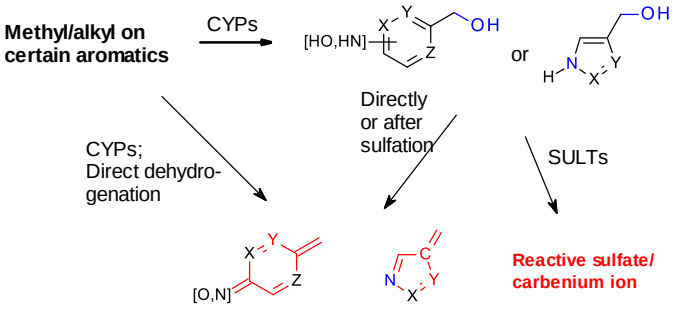

Figure 2. General scheme for methide formation.

Group B, score 5-7. Several SARMs can be put into this category, ranging from "Open 2,3-disubst thiophenes" to "Furans", both with a "7" scoring, in the upper end of the group. Other alerts that we have given this ranking are "Benzylic alcohol, activated", "Cyclopropylamines", "Allyl-H", and many more. In fact, this is the dominating SARM group with about 50 % of all severity designations. In a typical drug-like molecule there are most often several substructures that can generate RMs; one can just mention

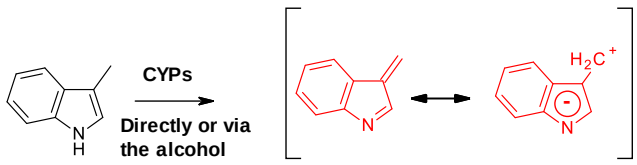

Figure 3. 3-Methylindole is the most well-known precursor of the fulvene-like methides.

the many clinical drug candidates that contain aromatic structures, most often benzene,[15] which is known for being able to generate reactive epoxides in addition to metabolism at the substituents. Could one possibly try to rank a benzene with no substituents? We have put "Naked phenyl" in the current group B with a score of "5".

Group C, score 1-4. These are the alerts that provide hardly noticeable nuisances in the drug toxicology machinery since they are omnipresent. They can probably cause problems, especially in combination with other SARMs. However, this is an area which must for now be considered to consist of "unknowns" and thus creating problems in any scoring attempts.

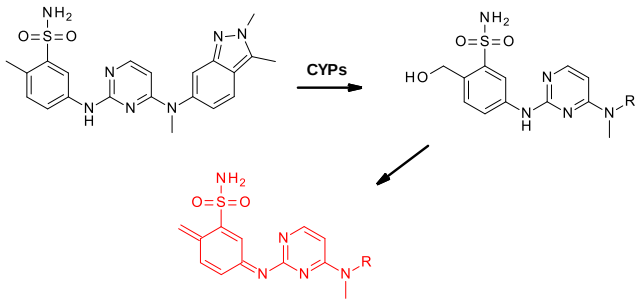

Figure 4. Bioactivation of pazopanib.

Taking one example from this category, one can mention an o-aryl alkyl ether, which along two paths can be metabolized to a phenol and then to quinones. Another is a catechol diether, which eventually may form a quinone, too. Generally speaking, SARMs in this group represent what remains when the major concerns from Groups A and B have been identified.

When running the set of druglike structures in the database ChEMBL through SpotRM, more than 90 % of the compounds have more than one SMARTS hit.[16] The majority of the alerts hit in this test belong to Groups B-C, which partly testifies to the purpose of SpotRM to give general warnings even of putatively minor importance. The fact that many drugs, classified by some based on clinical experience as "no-DILI",[17] become hits in SpotRM, i.e. false positives, goes in the same direction. At the same time, it might not be obvious how some of the relevant drugs got their "no-DILI" label. In many cases no-DILI can be linked to a low degree of metabolism/conversion, sometimes also helped by a low dose.

3. CONCLUSIONS

The simplest approach to avoid introducing reactive metabolite issues into the drug design process is to analyze structures for the presence of substructures chosen from a set of SARMs. In the current paper we have tried to take the concept one step further by introducing a Severity Score for SARMs. Time will tell whether this approach is useful.

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Awametox AB, Lilldalsvägen 17 A, SE-14461 Rönninge, Sweden. E-mail: info@awametox.com