Tracking Where the O's Go

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Electrophilic metabolites may be formed during the process of metabolism and can present a great stumbling block to investigational drug substances.[1] When reactive metabolites form covalent adducts to DNA, cytochrome P450 (CYP) enzymes, or proteins, the result ranges from mutagenicity to drug–drug interactions to idiosyncratic adverse drug reactions (IADRs). IADRs, which are a major reason for drug recall and/or restrictions in use, are triggered via haptens derived from the reaction of reactive drug metabolites with cellular proteins.[1] Being able to predict the sites and products of drug metabolism will go a long way to help design therapeutics immune to such degradation pathways. Hughes et al. provide great progress toward such a method.[2]

Assuming that an investigational therapeutic agent, immune to reactive metabolite formation, will be devoid of IADR risks, functional groups associated with reactive metabolite liability (structural alerts)[2] are generally excluded in drug design, when possible. However, there is no clear distinction as to when a particular functionality is viewed as a structural alert. For example, the vast majority of marketed drugs possess a phenyl ring, which is a structural alert, since its oxidative metabolism to the corresponding phenol metabolite proceeds via a reactive epoxide intermediate.[3] It is practically impossible to replace simple phenyl rings from the functional group inventory, and mankind would be deprived of numerous drugs if all phenyl-containing drugs had not been developed because of a structural alert designation.

Hughes et al.'s computational model can predict the likelihood of epoxidation and the corresponding site(s) of epoxidation on double bonds whether they are simple olefins or located within aromatic or heteroaromatic rings.[2] The foundation for the model is based on the XenoSite algorithm previously written by Swamidass and coworkers[5] to predict sites of oxidative metabolism by CYP enzymes, and recently applied to study intrinsic reactivity of electrophiles with the endogenous antioxidant glutathione.[6] The authors used more than 350 molecules possessing aromatic/heteroaromatic rings and/or double bonds in their chemical architecture to train the

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present model, and applied it to the retrospective prediction of epoxidation sites in structurally distinct small molecule drugs (e.g., carbamazepine, furosemide, sudoxicam, etc.), which are known to generate electrophilic epoxides and are associated with a significant incidence of IADRs. The ability to prospectively predict epoxidation products could prove incredibly valuable, especially in exploratory drug discovery, saving the need to synthesize molecules for optimization of *in vitro* pharmacology and absorption, distribution, metabolism, and excretion characteristics for the more promising candidates.

The potential for reactive metabolite formation on a structural alert depends on the binding pose of the molecule in the active site of the drug metabolizing enzyme (e.g., CYP), and subsequent positioning of the structural alert toward metabolism to a reactive species. To analyze this experimentally, potential drugs are incubated in human liver microsomes containing the CYP cofactor NADPH and an exogenously added nucleophile such as glutathione (GSH) to trap reactive metabolites. For example, in the course of discovery efforts leading to the discovery of taranabant (Figure 1), a selective and potent inhibitor of the cannabinoid-1 receptor, and a phase III clinical candidate for the treatment of obesity, the lead compound **1** revealed a high degree of covalent binding to human liver microsomes in a NADPH-dependent fashion, consistent with the formation of a protein- and glutathione (GSH)-reactive metabolite(s) through the action of CYP enzyme(s). Elucidation of the structure of the
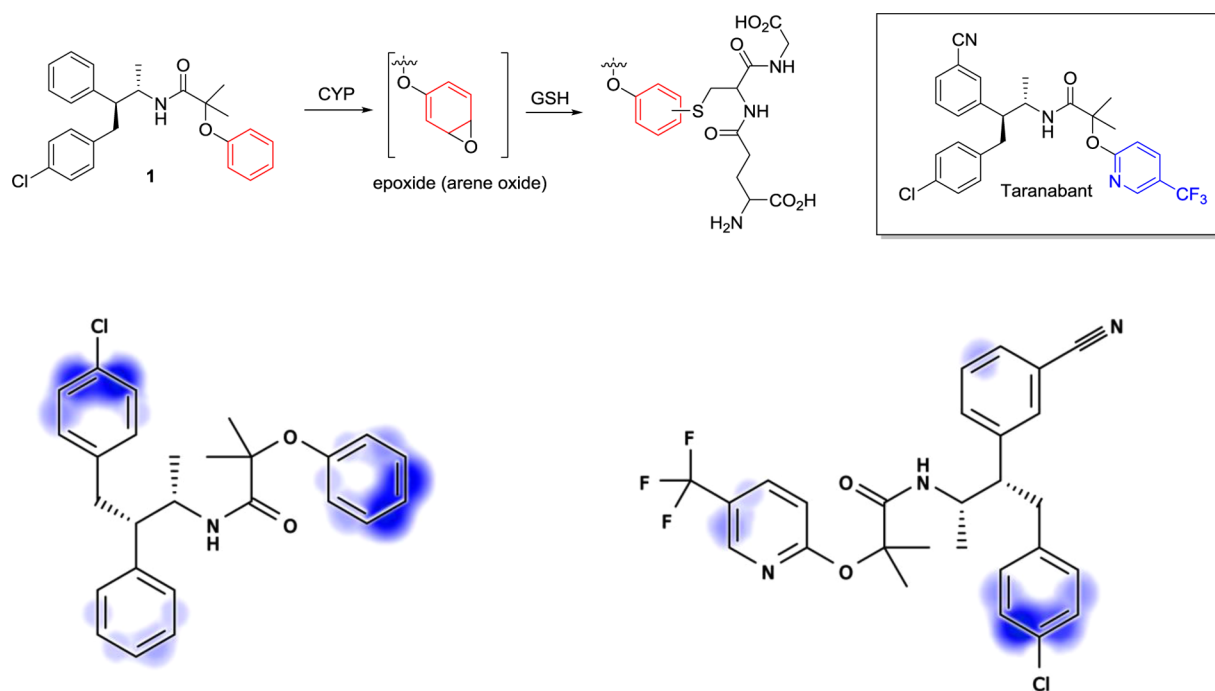
Figure 1. Eliminating the formation of reactive arene oxide metabolites in the cannabinoid-1 receptor antagonist program leading to the discovery of taranabant. Heat maps of the relative sites of potential epoxidation in both **1** and taranabant.
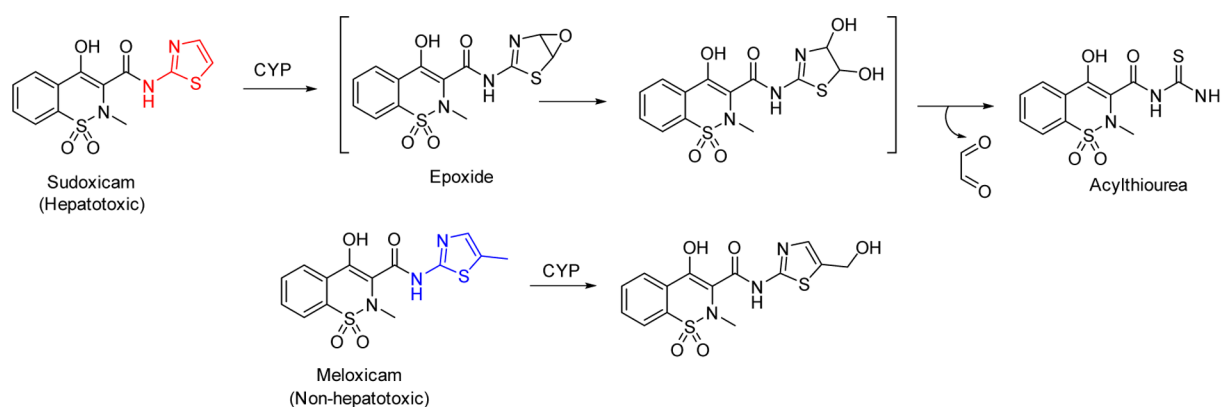
Figure 2. Differences in the metabolic fate of sudoxicam and meloxicam.

GSH conjugate suggested that the reactive metabolite was an arene oxide (epoxide) intermediate.[7] Through iterative medicinal chemistry design cycles, it was found that replacing the phenoxy ring with the trifluoromethylpyridyl ring afforded a product not metabolized to reactive epoxide species while retaining the potency and selectivity against the cannabinoid-1 receptor (taranabant).

As part of this First Reaction, the authors were asked to perform their analysis on taranabant. Gratifyingly, the computational model correctly predicts the site of epoxidation in **1** (Figure 1). Further, the structural modifications involving replacement of the phenyl group with the trifluoromethylpyridyl motif are predicted to be less susceptible to epoxidation. This case study demonstrates the great potential of computational methods to forecast and analyze potential metabolic products.

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This is not to say that the method is without its problems. A shortcoming of the computational model lies in the fact that it cannot predict whether epoxidation will be a major or a minor metabolic fate. It does not assign any quantitative estimate to the relative contribution of the epoxidation process compared to other metabolic events. This is highlighted in the

publication with the case study on sudoxicam and meloxicam, where only the former has liver toxicity. Both drugs contain 2-aminothiazole (a structural alert), but only sudoxicam is metabolized to the reactive acylthiourea through the epoxide intermediate) as a major metabolic fate in animals and human leading to its hepatotoxicity (Figure 2). A small but crucial addition of a methyl group at the C-5 position on the thiazole ring in meloxicam dramatically alters the metabolic profile such that, in human, meloxicam is primarily metabolized to an alcohol (off the C-5) methyl group. By comparison, thiazole ring epoxidation in meloxicam is a minor metabolic event.[8] The computational model successfully predicts thiazole ring epoxidation on both compounds; however, it assigns an equal probability for epoxide formation with both agents. In other words, the model, presently, cannot distinguish the possibility of metabolism occurring at a site other than the structural alert, and generating nonreactive products. Nonetheless, this model is an important step forward in predicting epoxidation in aromatic/heteroaromatic ring systems. It is possible that more comprehensive modeling of metabolism using these approaches could identify problematic molecules early in development, and guide appropriate modifications to avert potential IADRs due to reactive metabolite formation.

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