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Metabolic Activation and Hepatotoxicity of Furan-Containing Compounds

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ABSTRACT

Furan-containing compounds are abundant in nature, and many, but not all, have been found to be hepatotoxic and carcinogenic. The furan ring present in the chemical structures may be one of the domineering factors to bring about the toxic response resulting from the generation of reactive epoxide or *cis*-enedial intermediates, which have the potential to react with biomacromolecules. This review sets out to explore the relationship between the metabolic activation and hepatotoxicity of furan-containing compounds on the strength of scientific reports on several typical alkylated furans, synthetic pharmaceuticals, and components extracted from herbal medicines. The pharmacological activities as well as concrete evidence of their liver injuries are described, and the potential toxic mechanisms were discussed partly based on our previous work. Efforts were made to understand the development of liver injury and seek solutions to prevent adverse effects.

SIGNIFICANCE STATEMENT

This review mainly elucidates the vital role of metabolic activation in the hepatotoxicity of furan-containing compounds through several typical chemicals studied. The possible mechanisms involved in the toxicities are discussed based on collective literatures as well as our work. Additionally, the structural features responsible for toxicities are elaborated to predict toxicity potentials of furan-containing compounds. This article may assist to seek solutions for the occurring problems and prevent the toxic effects of compounds with furan(s) in clinical practice.

Introduction

Furan-containing compounds are plenty in food, perfumes, synthetic pharmaceuticals, natural herbal medicines, and environmental pollutants (Saunders et al., 1974; Zhou et al., 2004; Tundis et al., 2014; Knutsen et al., 2017; Delost et al., 2018). On the basis of structural features, these compounds could be briefly divided into furanoterpenoids (Fig. 1), furocoumarins (Fig. 2), benzofurans (Fig. 3), and substituted furan(s) (Fig. 4). In addition to the beneficial effects in application, numerous furan-containing chemicals have been reported to be toxic in rodents and/or humans, including liver injury, lung/renal toxicity, damage to nervous system, and the like (Ravindranath et al., 1986; Haller and Benowitz, 2000; Huang et al., 2020). Furans could also result in mechanism-based inactivation of cytochrome P450 enzymes (P450) and possibly cause drug-drug interactions when coadministered with other medicines (Cao et al., 2015; Fu et al., 2020).

Focused on the hepatotoxic aspects, drug-induced liver injury (DILI), responsible for approximately 25% of clinical drug safety failures in the clinic, has been classified into hepatocellular injury, cholestasis, or mixture of the two types. Hepatocellular DILI involves direct damage of the hepatocytes and is presented as the abnormally elevated level of serum transaminase, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Cholestasis results from the impaired bile excretion by hepatocytes and manifests as the increased level of γ-glutamyl peptidase, alkaline phosphatase, and total bilirubin (Shi et al., 2017; Norman, 2020). Proposed mechanisms associated with DILI include drug-induced mitochondrial dysfunction, inhibition of hepatic transporters, and the formation of reactive metabolites (Gómez-Lechón et al., 2016; Fraser et al., 2018).

Furan, a five-membered oxygen heterocyclic and aromatic compound, is listed as a possible human carcinogen (class 2B) by the National Toxicology Program and the International Agency for

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ABBREVIATIONS: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDA, *cis*-2-butene-1,4-dial; BSO, buthionine sulfoximine; dAdo, 2′-deoxyadenosine; DBL, *Dioscorea bulbifera* L.; DC, Dictamni Cortex; dCyd, 2′-deoxycytidine; DDE, DSB-derived *cis*-enedial; DEX, dexamethasone; dGuo, 2′-deoxyguanosine; DIC, dictamnine; DILI, drug induced liver injury; DMF, 2,5-dimethylfuran; DSB, Diosbulbin B; EDE, EEA-derived *cis*-enedial intermediate; EEA, 8-Epidiosbulbin E acetate; GSH, glutathione; 4-IPO, 4-Ipomeanol; KTC, ketoconazole; 2-MF, 2-methylfuran; MLM, mouse liver microsome; NAC, *N*-acetyl cysteine; NAL, *N*-acetyl lysine; NFT, nitrofurantoin; P450, cytochrome P450 enzymes; TA, teucrin A; dAdo, 2′-deoxyadenosine; dCyd, 2′-deoxycytidine; dGuo, 2′-deoxyguanosine.

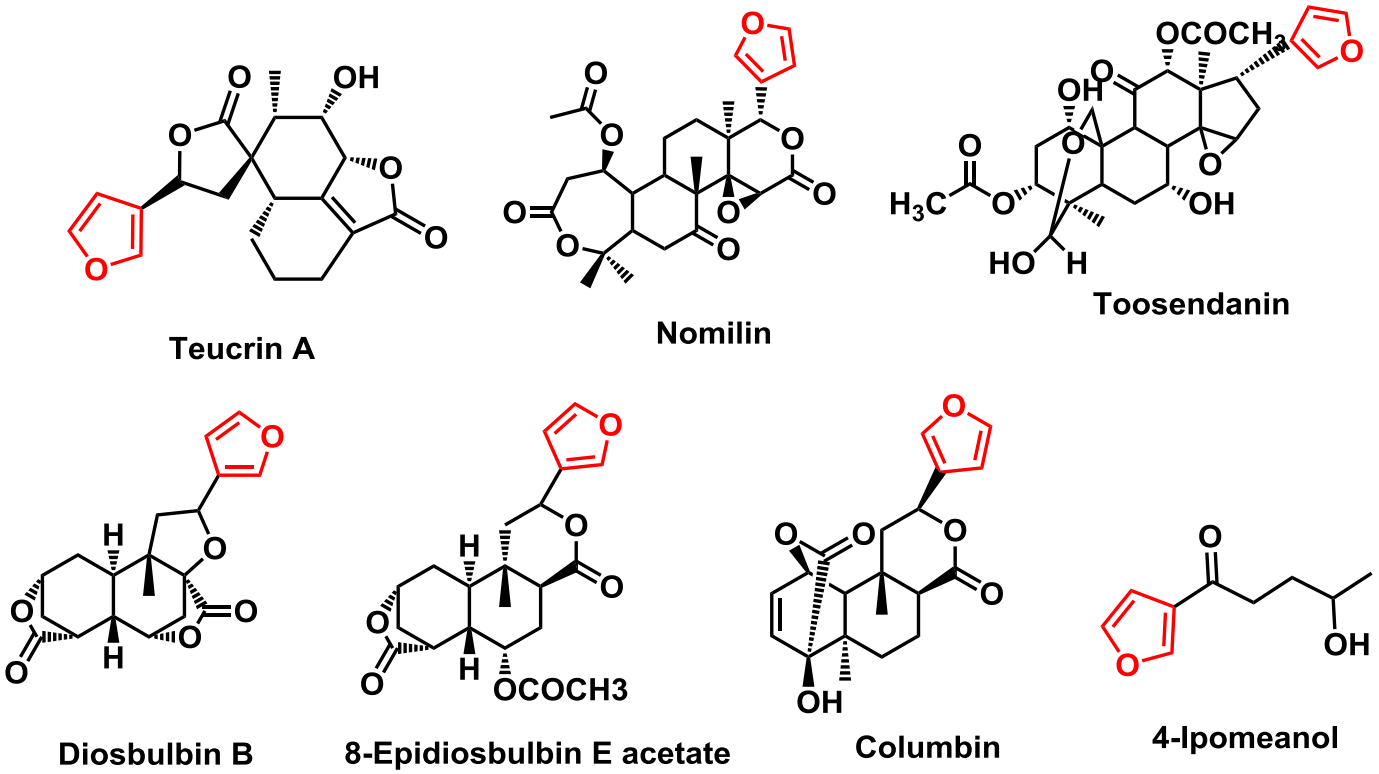


Fig. 1. Structures of common furanoterpenoids.

Research on Cancer because of its potential to induce carcinogenicity and liver toxicity in experimental animals (Peterson, 2013). It could be bioactivated by P450 to reactive metabolites through two pathways (Scheme 1): epoxidation or addition of the high valent iron-oxospecies to the π-system of the ring to form either an epoxide or a zwitterionic intermediate. The furan epoxide metabolite would further rearrange to form a *cis*-enedial or hydrate to nontoxic vicinal diols, and the zwitterionic intermediate can similarly rearrange to generate a *cis*-enedial. CYP2E1 is proven to be the dominant enzyme responsible for the oxidation of furan to *cis*-2-butene-1,4-dial (BDA) metabolite that is of low stability and high reactivity (Chen et al., 1995; Guengerich, 2003; Gates et al., 2012).

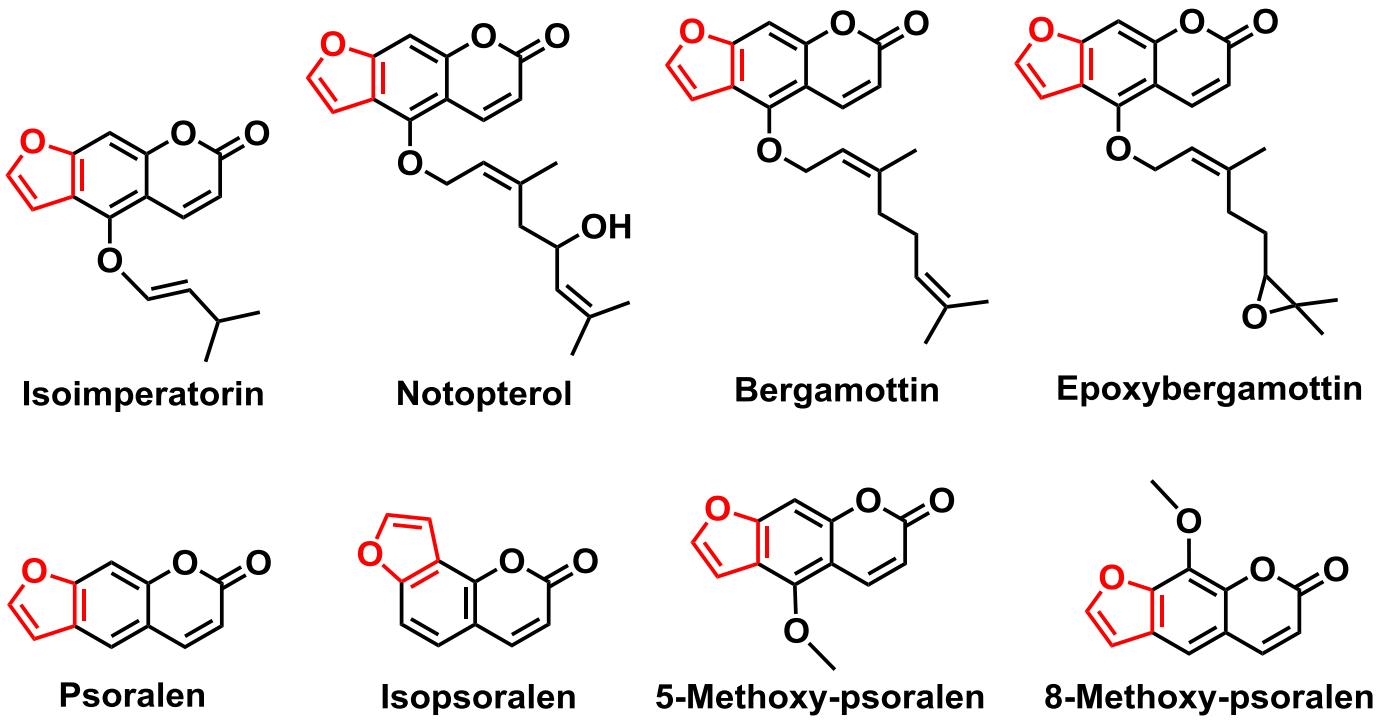


Fig. 2. Structures of typical furocoumarins.

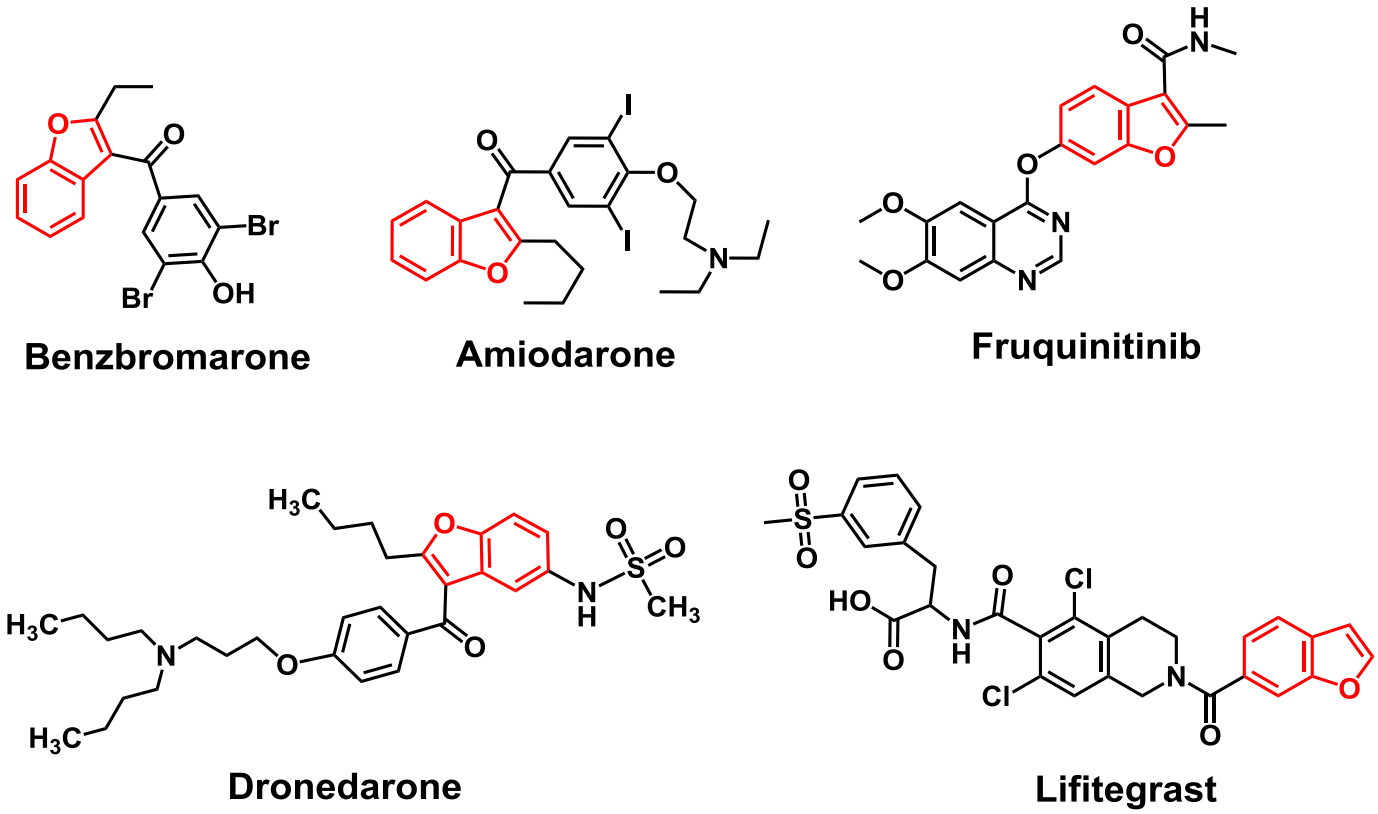


Fig. 3. Compounds containing benzofuran ring.

Trapping agents and relevant technologies applied to monitor the final products have been gradually developed to assist identification of BDA (Dalvie et al., 2015), which could react with glutathione (GSH) to form the mono- or bis-GSH conjugates in microsomal incubations (Peterson et al., 2005), and GSH-BDA can further crosslink with polyamines (ornithine, spermidine, and putrescine) to generate

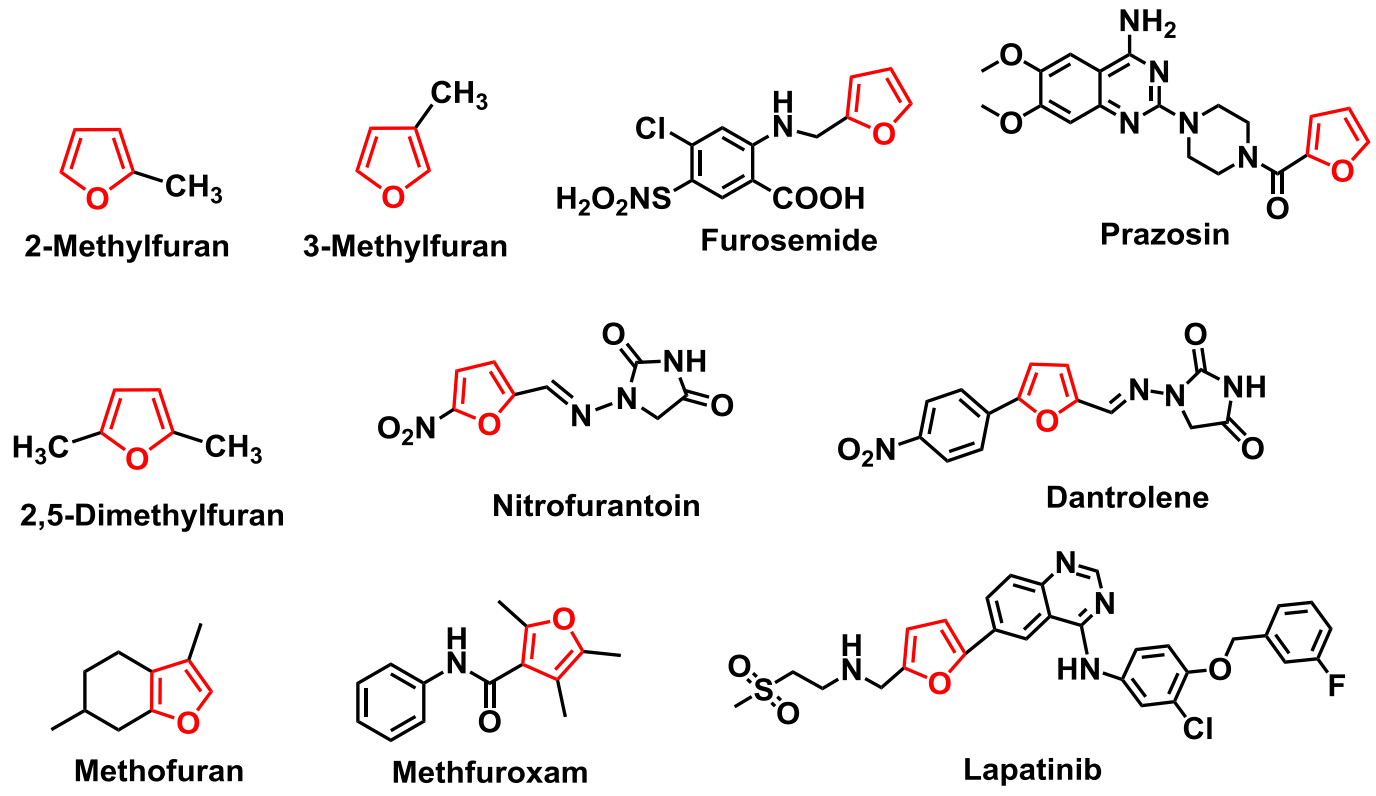
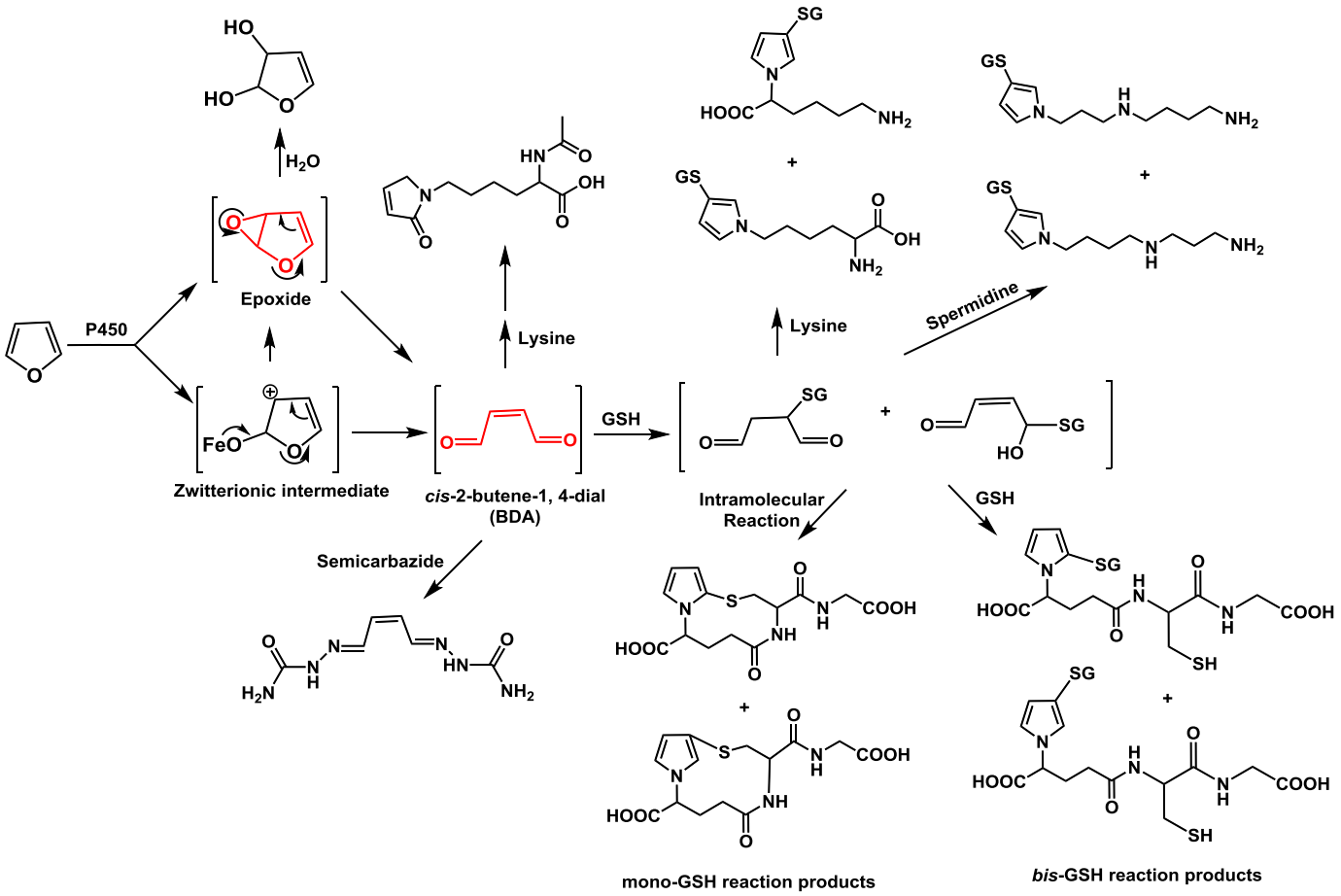


Fig. 4. Furans substituted with various group(s).



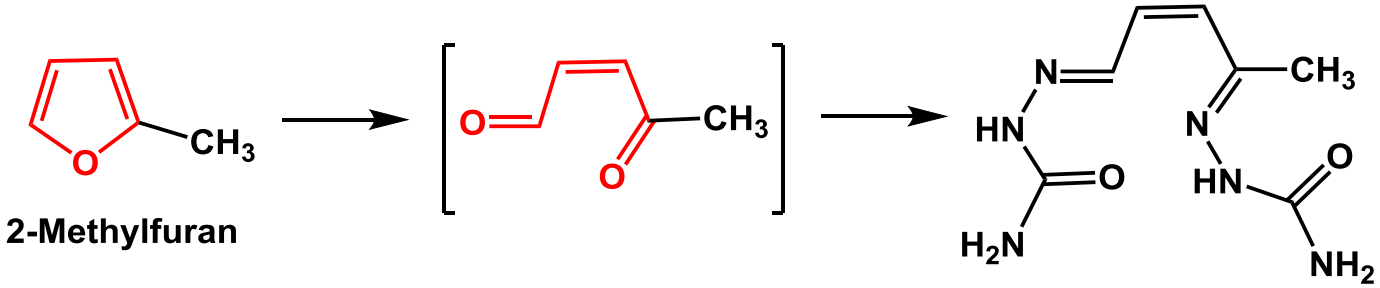
Scheme 1. Proposed metabolic oxidation of the furan ring to an epoxide or a *cis*-endione and subsequent reaction with other nucleophilic agents (Chen et al., 1995; Guengerich, 2003; Gates et al., 2012; Peterson, 2013).

stable metabolites in hepatocytes (Peterson et al., 2011). In addition, a sensitive and effective analytical platform using GSH and 4-bromobenzylamine as the trapping reagents to recognize the α,β-unsaturated dialdehyde has already been established by our research team (Wang et al., 2014). BDA is also capable of being trapped by *N*-acetyl lysine (NAL) and *N*-acetyl cysteine (NAC) with nucleophilic groups to obtain pyrrole derivatives (Gates et al., 2012). The high affinity to sulfhydryl and amino groups makes it possible that BDA could react with protein or nucleoside to form adducts (Byrns et al., 2004; Lu et al., 2009; Lu and Peterson, 2010). Developed novel approaches to separate and determine the covalently bound proteins incorporated with high-throughput proteomics and mass spectrometry have found that the target proteins of furan were primarily involved in mitochondrial energy production, redox regulation, and protein folding, of which the functional damage could disrupt cell homeostasis and cause cell death (Moro et al., 2012).

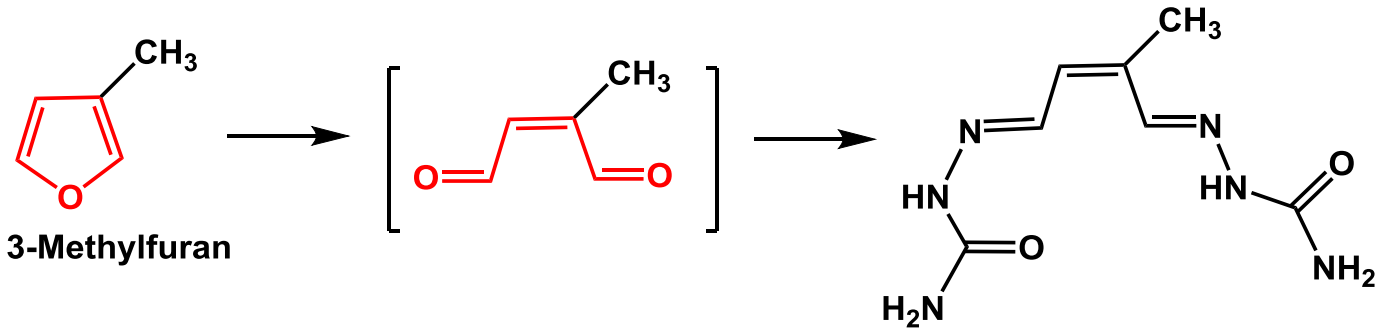
Based on previous studies developed with the furan ring, this review aims to explore the relationship of metabolic activation and hepatotoxicity caused by furan-containing compounds. In the next subsections, different and typical exogenous chemicals originated from synthesis or nature will be discussed according to our perception.

Methylfurans. Metabolic activation of the furan ring still plays a vital role in triggering toxicities when it is substituted by one or more methyl groups, such as 2-methylfuran, 3-methylfuran, and 2,5-dimethylfuran found concurrently in thermal processed food and fruit juices (Frank et al., 2020).

Liver is the primary toxic target organ of 2-methylfuran (2-MF), according to the histopathological and biochemical indicators of the male Fisher 344 rats after 28-day exposure (Gill et al., 2014). Centrilobular necrosis of the liver accompanied with the elevation of serum glutamic pyruvic transaminase and bronchial injury of the lung occurred in male Sprague-Dawley rats administered with 2-MF at a dosage of 100



Scheme 2. Trapping of the reactive acetyl acrolein formed in the oxidation of 2-methylfuran by semicarbazide in microsomal incubations (Ravindranath et al., 1984).



Scheme 3. Reactive 2-methyl-*cis*-2-butene-1,4-dial intermediate derived from 3-methylfuran formed in the CYPs mediated oxidation and conjugated with semicarbazide (Ravindranath et al., 1984).

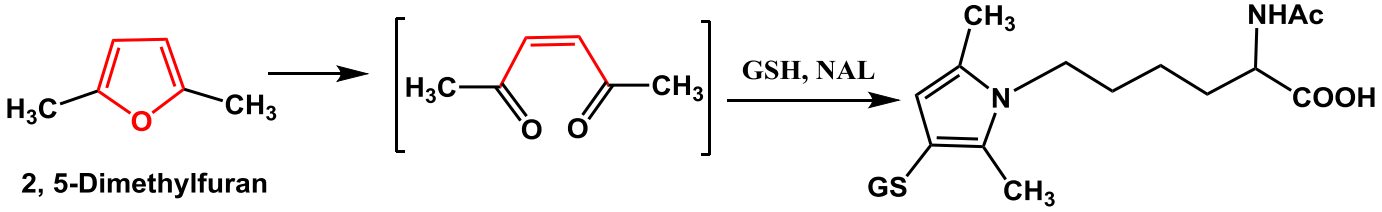
mg/kg (Ravindranath et al., 1986). 2-MF could be biotransformed to chemically reactive acetylacrolein catalyzed by P450 and further covalently bound to microsomal proteins and DNA (Scheme 2). Pretreating rats with phenobarbital (an inducer of P450) enhanced 2-MF–induced protein adduction and toxicity, whereas pretreatment with piperonyl butoxide (an inhibitor of P450) obtained the opposite result, indicating the vital role of the metabolic activation in 2-MF–induced hepatotoxicity (Ravindranath and Boyd, 1985). 3-Methylfuran could similarly be metabolized to 2-methyl-*cis*-2-butene-1,4-dial (Scheme 3) which reacted with hepatic and pulmonary proteins and caused toxic effects (Ravindranath et al., 1984). 2,5-Dimethylfuran (DMF), being developed to a biofuel and as another food contaminant, was reported to be clastogenic and genotoxic in mammalian hematopoietic cells (Fromowitz et al., 2012). It could be metabolically activated to epoxide or/and *cis*-enedial, followed by reacting with GSH to form the corresponding conjugates excreted in bile of rats, and the resulting GSH conjugate could be further captured using external NAL to generate the pyrrole derivative (Scheme 4) (Li et al., 2015). The reactive metabolite of DMF was able to modify the cysteine and lysine residues of proteins both in microsomal incubations and rats, which can be trapped by 4-bromobenzylmercaptan or 4-bromobenzylamine. Mild elevation of serum ALT was observed in mice administered DMF at 35 mg/kg, and the elevation continued with the increase of dose administered. Similar dose-dependent DMF-derived protein adduction was observed in mice (Wang et al., 2015).

4-Ipomeanol. 4-Ipomeanol (4-IPO), ipomeanine, 1-ipomeanol, and 1,4-ipomeadiol are all degradation products of furanosesquiterpenoid metabolites produced by sweet potatoes, which was reported to cause pulmonary toxicity in mice (Boyd et al., 1974; Burka and Wilson, 1976). Besides, 4-IPO was used to treat lung cancer but led to severe liver damage in patients (Rowinsky et al., 1993). The reactive metabolites of 4-IPO formed in situ were possible to alkylate the macromolecular constituents of the target tissues and induce toxic responses, and oxidation of the furan moiety may be indispensable based on the decreased toxicity of 4-IPO analogs with replacement of furan ring (Boyd and Burka, 1978; Boyd et al., 1980). Furthermore, different expression levels of P450 in specific tissues would influence the toxic target organ of 4-IPO, which was pulmonary in rodents mediated by CYP4B1, whereas liver in humans catalyzed by CYPs1A2, 2C19, 2D6, and 3A4 (Czerwinski et al., 1991; Baer et al., 2005).

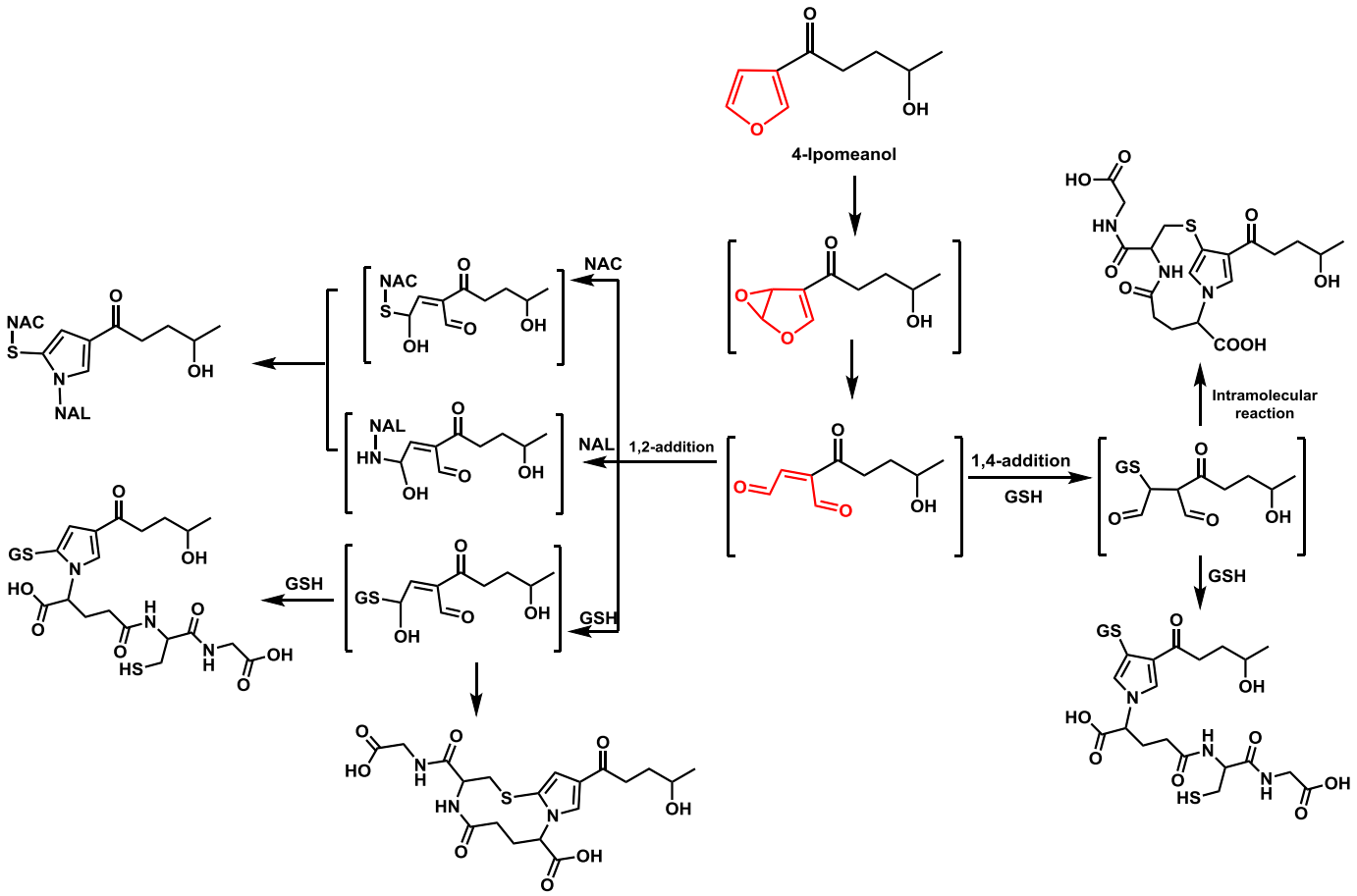
Two 4-IPO-derived GSH adducts were detected in incubational systems fortified with rat lung or liver microsomes and NADPH. The chemical structures of the reactive metabolites of 4-IPO have not been characterized because of the restriction of detectors. Disappearance of the UV absorbance of the furan ring reflected its oxidation (Buckpitt and Boyd, 1980). Four GSH conjugates monitored by acquiring the same molecular ions were found in the bile samples of rats exposed to 4-IPO and suspected to be derived from the conjugation of GSH with epoxides or α,β-unsaturated di-aldehyde intermediates (Scheme 5) (Alvarez-Diez and Zheng, 2004).

Teucrin A. Germander, one plant of the Lamiaceae family, has long been used to treat inflammation, gout, depression, indigestion and other diseases. It has also been employed for weight loss consumed alone or combined with camellia tea in 1991 (Larrey et al., 1992; Salah et al., 2006). Unfortunately, about 30 cases of liver injury have been reported in a short time, which resulted in the prohibition of the diet products (Castot A and Larrey D, 1992; Pauwels et al., 1992; Ben Yahia et al., 1993). Among the extract of germander, furanoditerpenoid-containing fraction was found to be toxic to rat hepatocytes (Lekehal et al., 1996). Teucrin A (TA), as the major component of the fraction, showed similar midzonal hepatic necrosis in mice and was deemed to be responsible for the toxicity of germander (Kouzi et al., 1994).

Toxic response was apparently attenuated when the hepatocytes were pretreated with piperonyl butoxide and enhanced with the treatment of sodium phenobarbital. Consumption of GSH in advance or blocking glutathione synthesis exacerbated the hepatotoxicity, suggesting the important role of GSH depletion in the hepatotoxicity induced by TA. It is most likely that the furan ring is oxidized by P450 to chemically reactive 1,4-enedial intermediate, which could react with the free sulfhydryl groups present in GSH or proteins (Scheme 6) to trigger adverse effects (Kouzi et al., 1994; Lekehal et al., 1996). The toxicity disappeared when the furan ring of TA was selectively reduced, confirming the hypothesis above (Kouzi et al., 1994). Besides, in the system containing TA, NADPH and yeast microsomes expressing CYP3A4, a metabolite was detected and another two metabolites were



Scheme 4. Bioactivation of 2,5-dimethyl furan and the subsequent reaction with GSH and NAL (Li et al., 2015).



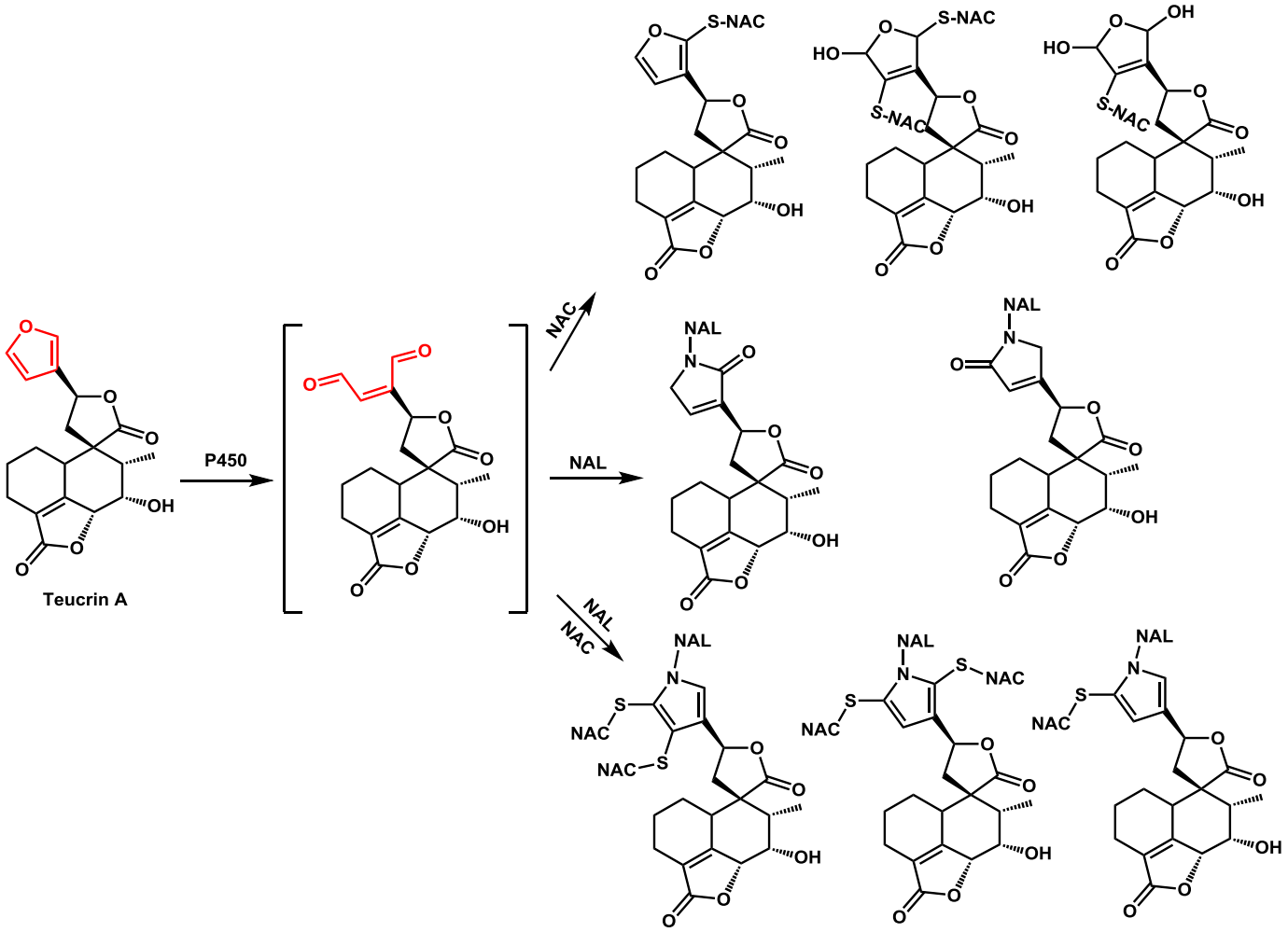
Scheme 5. Conjugation of GSH, NAC, or NAL with α,β-unsaturated di-aldehyde intermediates derived from 4-ipomeanol (Alvarez-Diez and Zheng, 2004; Chen et al., 2006).

generated after the addition of human microsomal epoxide hydrolase, whereas no product was observed in the absence of CYP3A4, indicating its necessity in the biotransformation of TA and the formation of a reactive epoxide (De Berardinis et al., 2000). To better clarify the toxic mechanism of TA, the oxidative enedial product of TA was synthesized and reacted with NAC or NAL methyl ester to form the corresponding stable pyrroles, which could also react with lysine-containing peptides to generate pyrrolinone derivatives (Druckova and Marnett, 2006). And subsequent immune enrichment of the adducted proteins with tailored antibody in rat liver manifested that the major targets of TA were mitochondrial, ER-associated proteins and enzymes involved in cell maintenance and small molecule metabolism (Druckova et al., 2007).

Diosbulbin B. *Dioscorea bulbifera* L. (DBL), which is generally called "Huang-Yao-Zi" in Chinese, is one member of the yam family *Dioscoreaceae* (Li et al., 2000). DBL is used to treat carbuncles, lung abscesses, breast lumps, and goiter, and also performs as the antifeedant, anti-inflammation, and antisalmonellal agent (Demetzos et al., 2001; Cifuente et al., 2002; Gao et al., 2002; Teponno et al., 2006). Nevertheless, the long-term and excessive consumption of DBL could cause severe liver damage in patients, and the ethyl acetate fraction of ethanol extracts showed the greatest toxicity (Wang et al., 2010). Lipophilic components, possibly furanoditerpenoids, are answerable for DBL-induced chronic and acute liver injuries (Li et al., 2020).

Diosbulbin B (DSB) is the most abundant diterpene lactone isolated from DBL and the major active component in anticancer action (Wang et al., 2012). It was reported to be responsible for the hepatotoxicity caused by DBL (Wang et al., 2011). Oral administration of DSB (32 mg/kg) for 12 consecutive days would provoke liver damage manifested as the increased activity of serum AST/AST/alkaline phosphatase and the swelling of hepatocytes. Besides, the levels of vital antioxidant enzymes and antioxidants in liver tissues (superoxide dismutase, catalase, glutathione *S*-transferase, and GSH) are all decreased, suggesting the occurrence of liver oxidative stress (Ma et al., 2014), which is similar to the toxic behaviors of DBL. After administration for 28 days in mice, DSB disordered the synthesis and transport of bile acid, promoting hyperbilirubinemia (Ji et al., 2020). Ye and coworkers' study showed that mitochondria are the target organelles for the hepatotoxicity of DSB in L-02 cells, and accumulation of reactive oxygen species can induce mitochondrion-dependent apoptosis, which probably plays a key role in DSB-induced hepatocellular injury (Ye et al., 2019).

Our previous study has provided clear evidence that the furan ring of DSB was metabolized to a *cis*-enedial intermediate in vitro and in vivo, which reacted with GSH, NAC, or NAL to form the corresponding stable pyrrole or pyrroline derivatives (Scheme 7). CYP3A was the primary enzyme to catalyze the metabolic activation, and the presence of ketoconazole (KTC, a specific inhibitor of CYP3A) decreased the formation of the reactive metabolite in microsomal incubations (Lin et al., 2014). To further determine the role of furan moiety in DSB-induced liver injury, tetrahydrofurano-DSB was synthesized to develop comparative studies in vivo. The saturation of the furan ring was found to reduce the hepatotoxicity of DSB. Furthermore, pretreatment of mice with KTC significantly increased the area under the curve of plasma DSB, reduced hepatic GSH depletion, and decreased susceptibility of

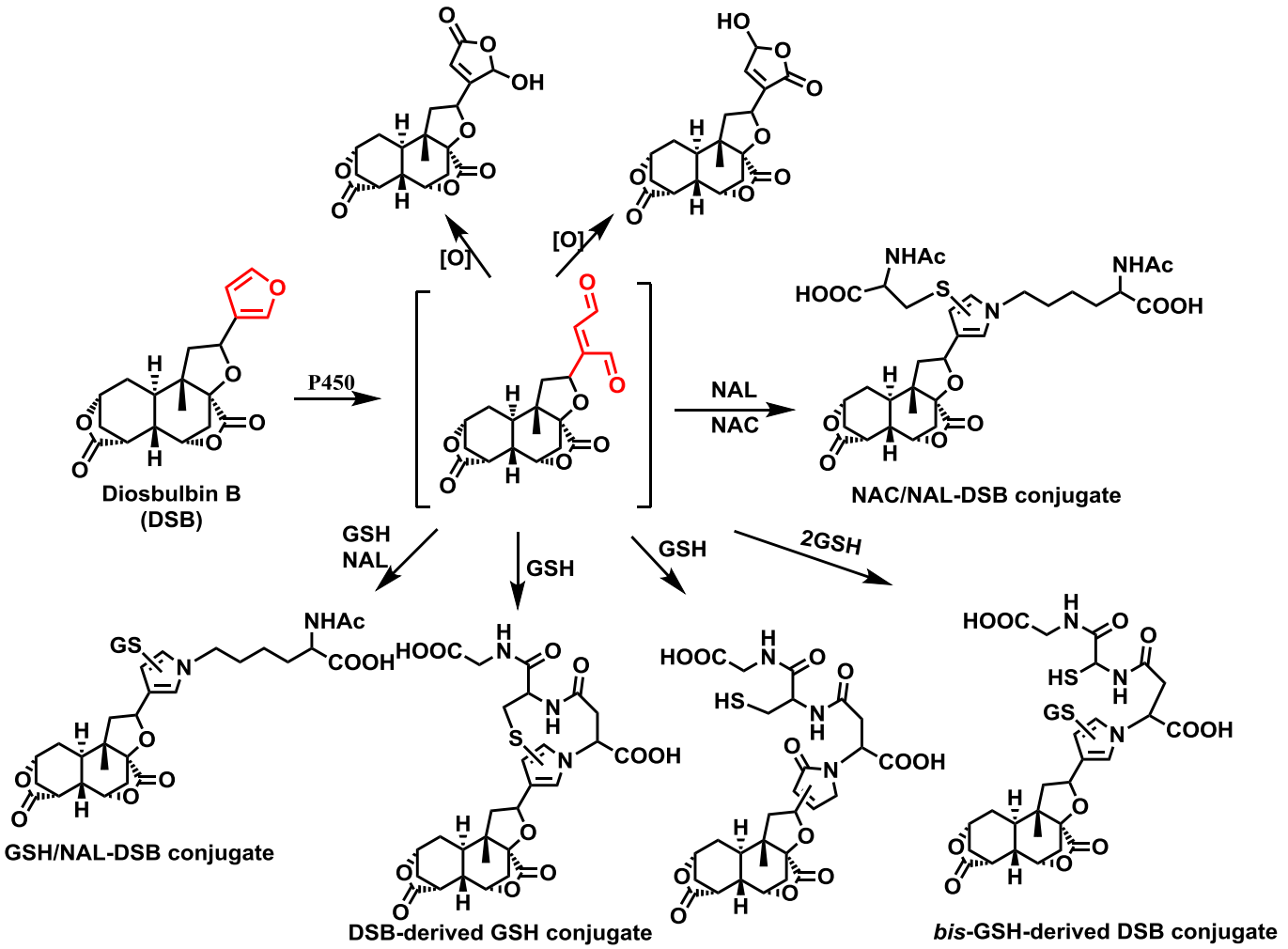


Scheme 6. Proposed oxidation of teucrin A and formation of NAC/NAL conjugate (Druckova and Marnett, 2006; Peterson, 2013).

animals to the hepatotoxicity of DSB. The findings indicate the reactive intermediate rather than the parent drug unleashed the toxicities (Li et al., 2016). Cytotoxicity evaluation of DSB was performed in P450-free NIH3T3 cells, primary rat hepatocytes, HepG2 and L02 cells of high CYP3A4 expression and wild-type. The results also suggest the close relationship between cytotoxicity and CYP3A4-mediated generation of reactive metabolites (Jiang et al., 2017).

Among the mechanisms proposed for the furan-related toxicities, protein modification has been considered to be an important part (Moro et al., 2012; Phillips et al., 2014). Thus, we developed a bromine-based analytical technique to explore the interaction of the reactive intermediate of DSB with proteins. Cysteine and lysine residues of mouse liver microsomal proteins reacted with the *cis*-enedial intermediate to form three different types of protein modifications (Scheme 8). The protein adduction was time- and dose-dependent in DSB-treated mice. Pretreatment of animals with KTC decreased the crosslink, whereas pretreatment with dexamethasone (DEX) or buthionine sulfoximine (BSO, an inhibitor of GSH biosynthesis) provided the opposite result, further illustrating the vital role of the metabolic activation in the reaction between DSB and proteins (Wang et al., 2017). This makes us speculate that modification of vital cellular proteins by DSB-derived *cis*-enedial (DDE) would disturb the normal functions and trigger liver injury. Facilitating the recognition of the DDE-derived protein adduction, we further successfully raised the polyclonal antibodies with high titers in rabbits immunized with the antigen prepared by reaction of KLH with DDE. Immunoblot analysis of the liver homogenates obtained from mice given different doses of DSB showed that much more intense protein bands were observed in the samples from 200 mg/kg DSB-treated mice than that from mice administered with 150 mg/kg DSB, similar to the dose-dependent hepatotoxicity observed above (Hu et al., 2018).

DNA covalent binding has been thought to be another vital action to trigger toxicities. BDA, the reactive metabolite of furan, was reported to be able to attack the exocyclic and endocyclic nitrogens of 2′-deoxycytidine (dCyd), 2′-deoxyguanosine (dGuo), and 2′-deoxyadenosine (dAdo) to form diastereomeric oxadiazabicyclooctaimine adducts (Gingipalli and Dedon, 2001; Byrns et al., 2002). To explore the possibility of interaction between DSB and DNA, DDE obtained by dimethyldioxirane oxidation reacted with dCyd, dGuo, and dAdo to obtain the corresponding adducts. However, the dAdo and dGuo adducts rapidly dehydrated to form the etheno adducts, which are the major forms existing under physiologic conditions, and the dCyd adducts preferred to exist as the cyclic hemiacetal by ring closure (Scheme 9). In the incubations fortified with calf thymus DNA as a model, the three adducts were also detected in the products after enzymatic hydrolysis, indicating the modification of DNA in vitro. Furthermore, the reactive aldehyde functionality present in etheno-dGuo



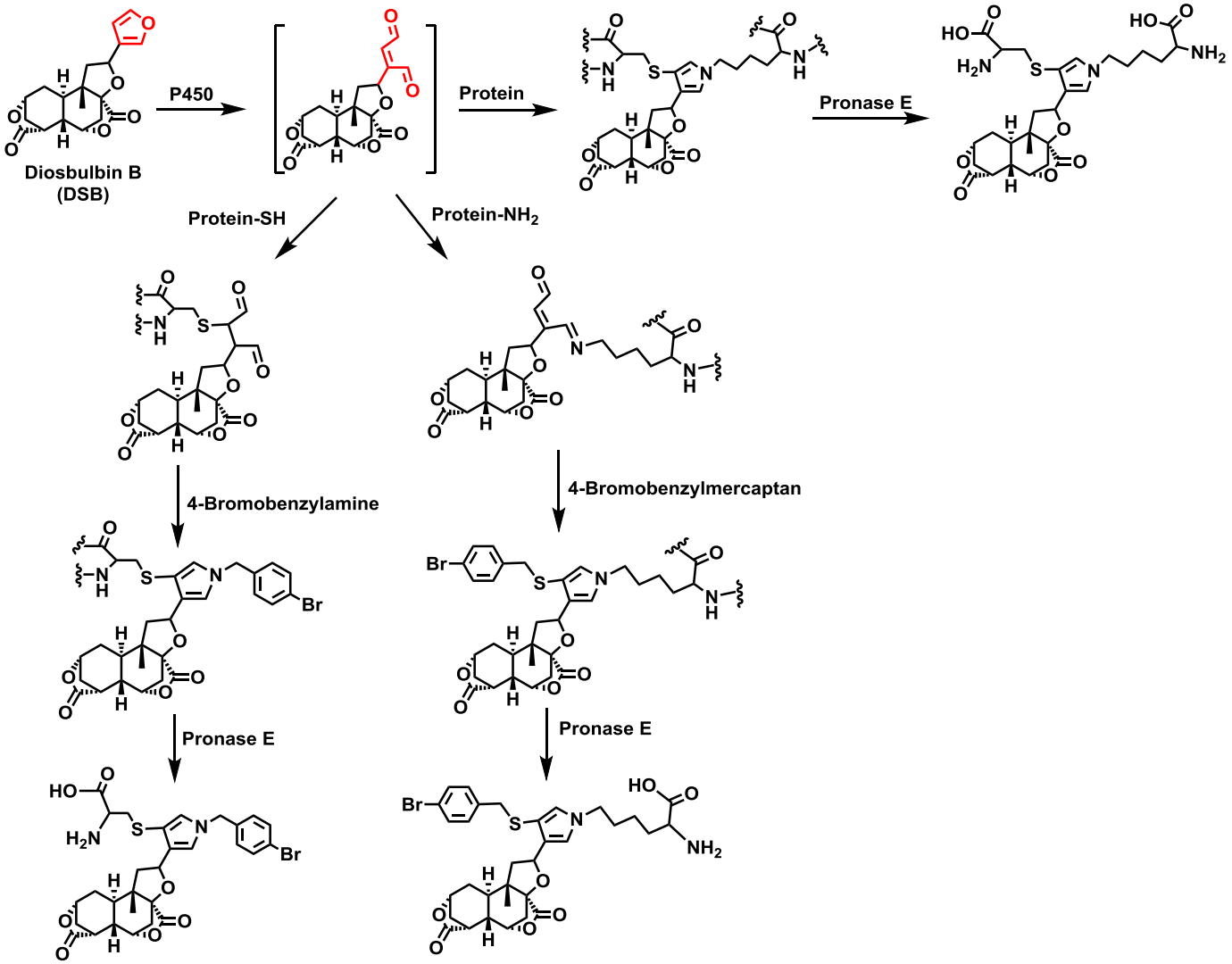
Scheme 7. Proposed metabolic activation of DSB mediated by P450 enzymes and the following reaction with GSH, NAC, and/or NAL (Lin et al., 2014).

and etheno-dAdo adduction may further react with nucleophiles of protein and/or DNA to cause DNA damage, contributing to the toxicities of DSB (Lin et al., 2019). In vivo evidence is needed to ensure the participation of DNA adduction in the toxicities of DSB. Polyamines, biogenic amine, and amino acid were found to react with DDE in rat liver microsomal incubations and primary hepatocytes, partly contributing to DSB-induced time- and concentration-dependent apoptosis and cell death (Zhang et al., 2020).

8-Epidiosbulbin E acetate. 8-Epidiosbulbin E acetate (EEA) is another diterpenoid lactone isolated from DBL in 1984 (Murray et al., 1984). Unexpectedly, EEA is the most abundant diterpenoid in some DBL, and its contents depend on the commercial source (Lin et al., 2015). Our previous studies found that the mice administered with EEA-containing DBL extracts showed 7.4- and 10.7-fold higher serum ALT and AST activities (ALT: 9128 ±3487 U/L; AST: 7189 ±4560 U/L) than those of the mice treated with EEA-free DBL extracts (ALT: 1093 ±322 U/L; AST: 613 ±194 U/L) (Lin et al., 2015).

The furan moiety was proven to be metabolized to EEA-derived *cis*-enedial intermediate (EDE) in vitro and in vivo, which was subsequently trapped by GSH and/or NAL to offer six cyclic GSH/NAL conjugates (Scheme 10), and CYP3A4 was the dominant enzyme to catalyze the process (Lin et al., 2015). Serum ALT and AST levels of EEA-treated mice presented time- and dose-dependent elevations. Local spotty necrosis and inflammatory cell infiltration were observed in the liver of EEA-treated mice (100 mg/kg for 36 hours). Pretreated with KTC reversed the elevations of serum transaminases and the pathohistological changes resulting from EEA exposure, suggesting the importance of the metabolic activation mediated by CYP3A4 in EEA-induced liver injury. Besides, EEA was accumulated in the circulation after KTC pretreatment, whereas the hepatotoxicity was not intensified, indicating parent EEA was not really toxic. Tetrahydrofurano-EEA was synthesized for the determination of the role of furan ring in EEA toxicity. The reduced EEA showed no hepatotoxicity in mice given the same dose of EEA, illustrating that unsaturation of the furan moiety is essential for the liver injury caused by EEA (Lin et al., 2016a).

In the microsomal incubations supplemented with GSH and EEA, two pyrrole and one pyrrolinone derivatives (A1-A3, Scheme 11) were detected and characterized by chemical synthesis to better understand the mechanisms of toxicities. The levels of A1–A3 manifested as the order of DEX-induced mouse liver microsomes (MLMs) > MLMs > KTC-treated MLMs, in accordance with our work mentioned above that CYP3A was the primary enzyme responsible for the bioactivation of EEA. The three adducts were all detected in the liver samples of mice given EEA, and their formation was time- and dose-dependent. Pretreatment with BSO increased cysteine-/lysine-based protein adduction and somewhat



Scheme 8. Proposed protein modification derived from *cis*-enedial intermediate resulting from metabolic activation of DSB (Wang et al., 2017).

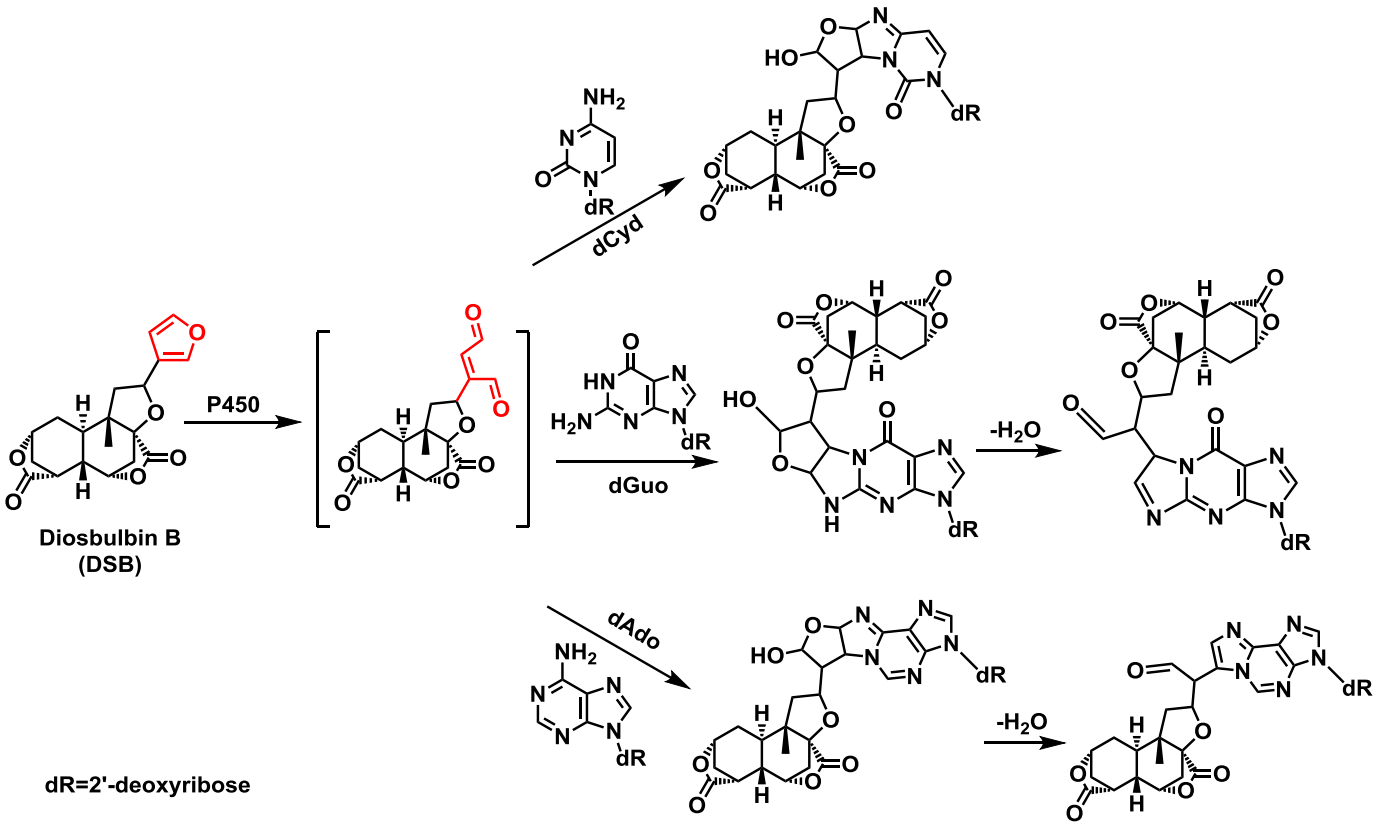
reduced GSH-/lysine-based protein adduction, indicating GSH depletion made the cysteine residues easier to be attacked by the reactive metabolites formed in the metabolic activation, resulting in hepatotoxicity through protein modification (Lin et al., 2016b). Polyclonal antibodies with high selectivity to recognize EDE-derived protein adducts were successfully prepared, allowing us to enrich and identify the target proteins modified by EDE (Zhou et al., 2020).

Like DSB, the reactive metabolite of EEA could also react with the exocyclic and endocyclic nitrogens of base monomer (dCyd, dGuo and dAdo, Scheme 12) to produce stable adducts and modify the dGuo, dAdo, and dCyd groups of calf thymus DNA. The potential to modify DNA may be another risk factor to cause EEA-induced hepatotoxicity and genotoxicity (Lin et al., 2019).

Dictamnine. Dictamnine (DIC) is a natural furan-quinoline alkaloid widely distributed in Dictamni Cortex (DC), Zanthoxylum armatum, and Toddalia asiatica (Zhu et al., 2019; Huang et al., 2020; Lin et al., 2020), showing antibacterial, antifungal, anticancer, and vascular-relaxation activities (Guo et al., 2008; Wang et al., 2018). Over 60 severe, even fatal, hepatotoxicity cases after ingesting DC have been reported worldwide, and DIC, as the major bioactive component, was speculated to be correlated with DC-induced liver injury. Oxidative stress injury and nuclear factor-*k*-gene binding (NF-κ,B) mediated inflammation were reported to be involved in DIC-induced acute liver injury in mice (Lin et al., 2021).

Study on the metabolism of DIC in respective systems containing liver microsomes across various species found that the furan ring could be oxidized to 2,3-epoxide primarily mediated by CYP3A4 (Wang et al., 2016). The epoxide intermediate reacted with NAC, GSH, or cysteine residues of proteins to open the ring and generate the corresponding conjugates and adducts in vitro and in vivo, which may be responsible for its toxicities (Feng et al., 2016; Lin et al., 2021). To evaluate the role of metabolic activation in DIC-induced liver injury, primary human hepatocytes, L02, HepG2 cells were chosen as the models. Cytotoxicity was increased in DEX-induced cells and decreased in KTC-inhibited ones. In addition, KTC pretreatment significantly lowered ALT and AST activities in media, alleviated DIC-induced hepatocyte degeneration and congestion, and DEX caused medium hepatocyte degeneration compared with the slight extent in control groups, suggesting the close relationship between the epoxidation and toxicity (Li et al., 2018).

Furthermore, DIC has also shown time- and dose-dependent hepatotoxicity in mice. Administration of DIC (150 mg/kg, 24



Scheme 9. Proposed reaction of DSB-derived *cis*-enedial with DNA bases (Lin et al., 2019).

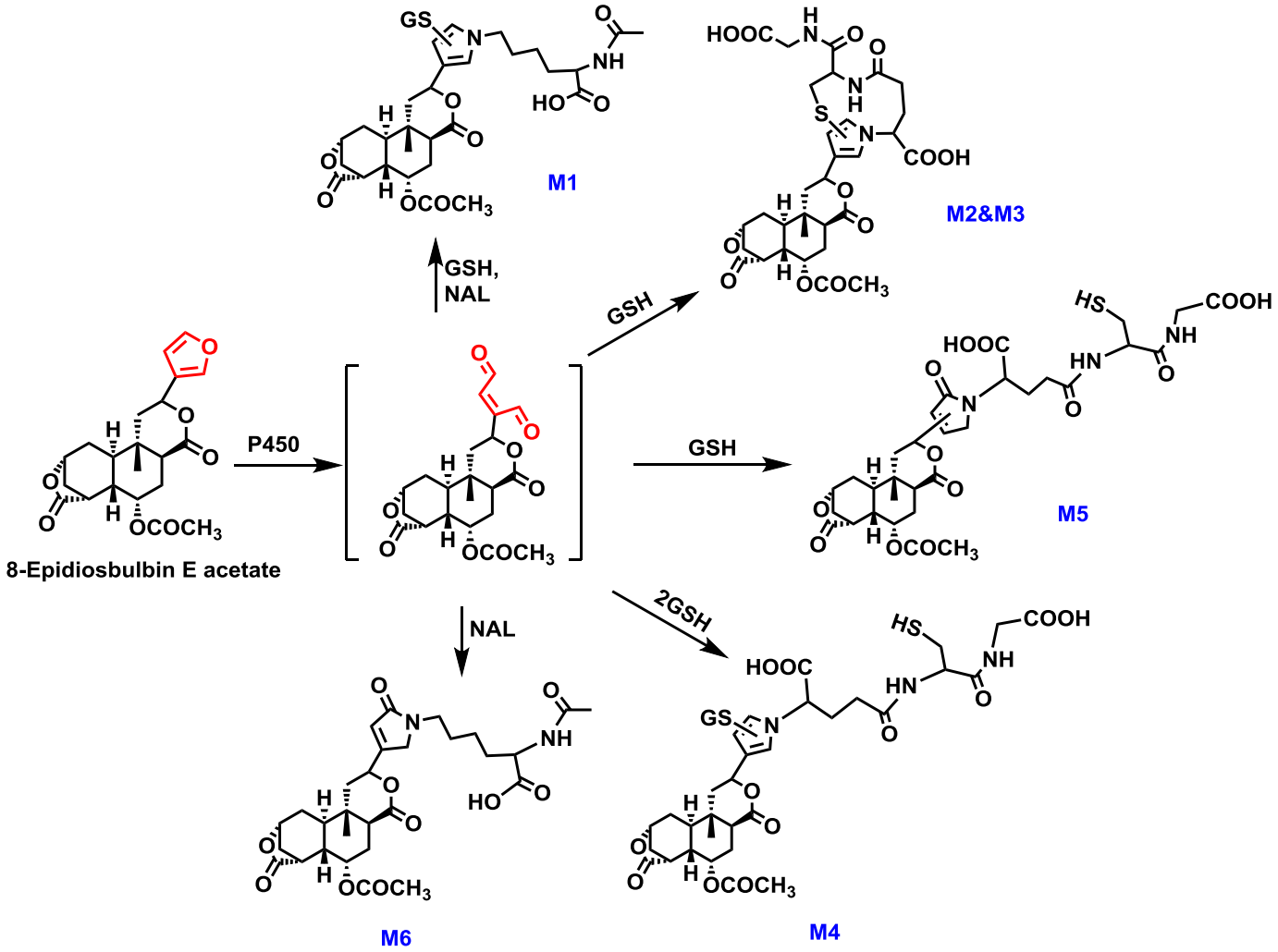
hours) resulted in elevation serum ALT and AST and liver necrosis around the central vein, along with the occurred submassive hydropic swelling and hepatocyte ballooning degeneration at 6 hours and 12 hours, which was reversed by KTC pretreatment. BSO pretreatment markedly increased the susceptibility of animals to the hepatotoxicity of DIC at a subtoxic dose (100 mg/kg), indicating GSH in part protected protein against the attack of the reactive intermediate. Synthetic 2,3-dihydro-DIC showed almost no toxicity compared with DIC at the same dosage, further demonstrating the vital role of the metabolic activation of furan ring in triggering hepatotoxicity. Moreover, fourfold increase in the area under the curve of plasma DIC was observed in KTC-pretreated animals in accompany with the reduction of DIC-induced hepatotoxicity, suggesting parent DIC is not the direct killer (Shi et al., 2019).

In addition to DIC, other furanoids abundant in ethanol extract of DC, such as obakunone, fraxinellone, and limonin, were also found to be biotransformed to reactive epoxide or *cis*-enedial metabolites. These accumulated reactive species cooperatively attributed to the reported liver damage (Huang et al., 2020).

Nitrofurantoin. Nitrofurantoin (NFT), composed of a nitrofuran group and a hydantoin side chain, is primarily used to treat the acute uncomplicated lower urinary tract infection and prevent the recurrence of urinary tract infections (Cunha, 1988; Gupta et al., 2011). It is the only one of the nitrofuran family currently in use in human medication and is available as an oral formulation only (Wijma et al., 2019). Compared with other antimicrobial drugs, there are few drug-resistant cases of NFT reported in clinic, promoting its continuous application (Guay, 2001; Araújo et al., 2011). However, the long-term use of NFT would trigger adverse drug reactions with different levels, such as diarrhea, dizziness, serious lung toxicity, neurotoxicity, and hepatotoxicity (Tan et al., 2012; Stock, 2014; Ramadas et al., 2018; Batzlaff and Koroscil, 2020).

NFT-induced liver injury is implicated as acute hepatitis, granulomatous reaction, cholestasis, autoimmune-mediated hepatitis, and chronic active hepatitis, even resulting in fulminant liver failure and death (Akšamija et al., 2009; Kiang et al., 2011; Sargõn et al., 2012; Sakaan et al., 2014; Sorin et al., 2016). Acute liver damage is rare and may occur days or weeks after exposure to NFT, causing the increased level of liver function enzymes and elevated percentage of eosinophils. Chronic hepatotoxicity generally presents months to years after initiation of long-term prophylactic therapy, accompanied by initial fatigue and weakness and followed by dark urine and jaundice (Sargõn et al., 2012; Sakaan et al., 2014).

The serological and pathologic features of 57 similar cases of chronic active hepatitis indicate the participation of autoimmunity mechanism (Sherigar et al., 2012). $CD8^+$ cytotoxic T cells, only found around the areas of necrosis, played a vital role in mediating NFT-induced liver damage (Kelly et al., 1998). Furthermore, reactive oxygen species, produced during the redox cycling in which nitro group is reduced to a nitro anion radical, would initiate oxidative stress and result in damage to hepatocytes (Moreno et al., 1984; Minchin et al., 1986). From the perspective of structure, we designed and synthesized nine probe compounds with various modifications to explore the correlation between toxicity and the hydantoin moiety, nitro group, and the furan ring. The nitroso and hydroxylamine intermediates formed by the reduction of the nitro group (Scheme 13) were responsible for GSH depletion and cytotoxicity induced by NFT (Li et al., 2019).

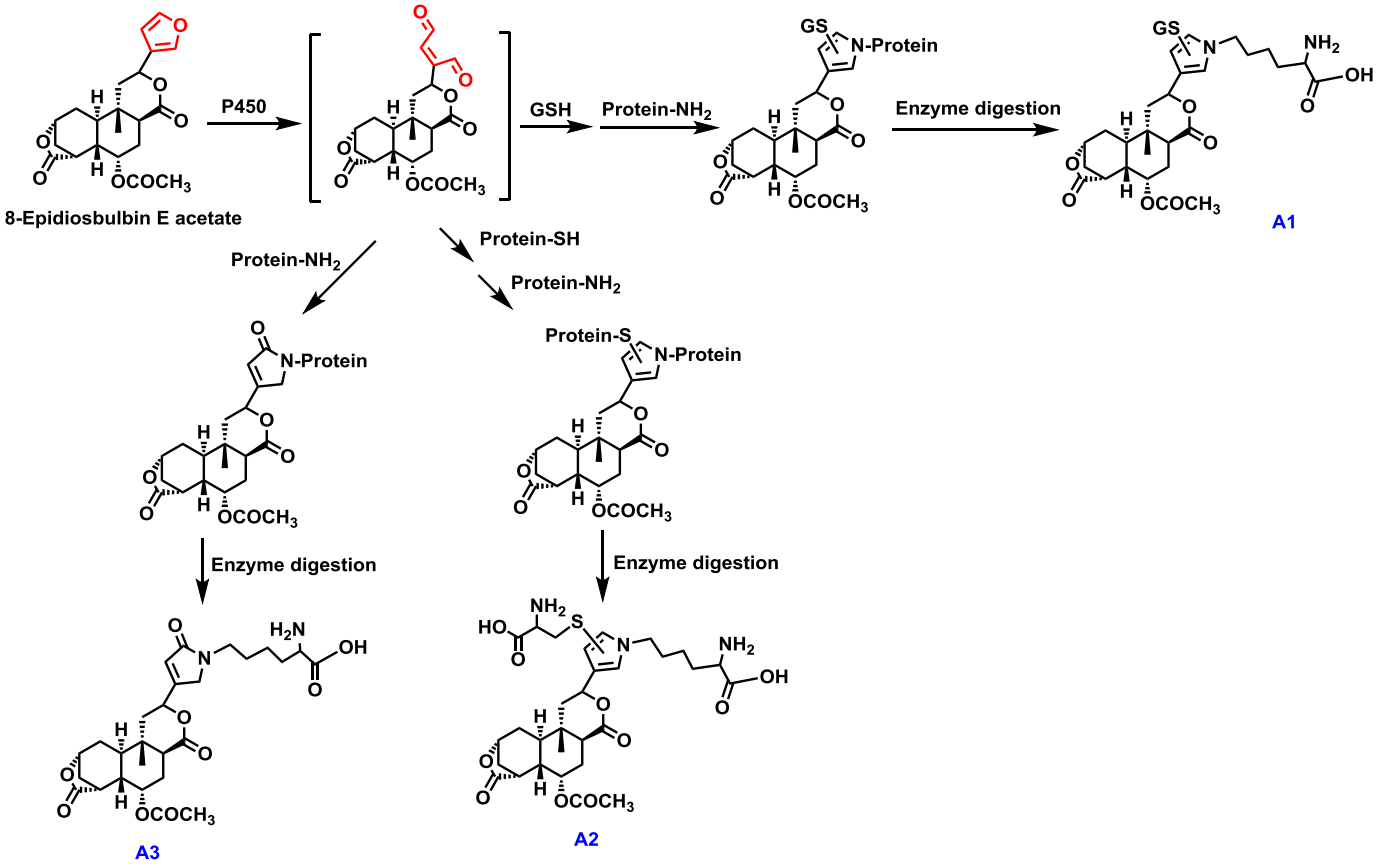


Scheme 10. Proposed metabolic activation of EEA mediated by P450 enzymes and the formation of GSH and/or NAL conjugates (Lin et al., 2015).

Besides, GSH depletion can be further attributed to the oxidative stress mentioned above (Rossi et al., 1988). Although reductive metabolism of NFT has been illustrated to be the major pathway (Aufrère et al., 1978), other possible biotransformations also need to be elucidated. In another study, an oxidative metabolic pathway (Scheme 14) of NFT mainly mediated by CYP3A5 and CYP2A6 was proposed. An epoxide intermediate was generated in microsomal incubations and subsequently underwent denitration when being attacked by thiols (GSH), possibly contributing to NFT-related toxic actions (Li et al., 2017).

TM5441. TM5441, an oral plasminogen activator inhibitor-1 inhibitor, could prevent high-fat diet-induced body weight gain and systemic insulin resistance and adipocyte injury in mice (Piao et al., 2016). No side effects related to TM5441 have been reported so far, but an internal study of Lang et al. (Lang et al., 2020) demonstrated that 7-day consecutive administration of TM5441 caused the elevations of serum ALT and AST in mice. In vitro and in vivo studies indicated that the furan ring of TM5441 may be metabolized to a *cis*-butene-1,4-dial intermediate, which reacted with GSH by 1,2- or 1,4-addition, followed by reacting with inter- or intramolecular amino group to form corresponding pyrrole derivatives (Scheme 15). CYP3A4 was proven to be the major enzyme participating in the generation of the reactive metabolite. Besides, there was no obvious increase of serum ALT and AST activities in mice administered tetrahydrofurano-TM5441, suggesting the vital role of furan ring in the development of the observed liver injury. Furthermore, the acylglucuronide derivative generated in the metabolism also depleted GSH to give rise to TM5441-*S*-acyl-GSH adduct, possibly contributing to the hepatotoxicity as well.

Prazosin and furosemide. Bioactivation of furan ring is vital in evoking hepatotoxicity in rodents or humans as mentioned above, but sometimes the bioactivation liability is lower than expected. Prazosin with a 2-substituted furan ring is used for the treatment of hypertension and post-traumatic stress disorder. Oxidation of the furan ring to enedione intermediate is the major metabolic pathway in vivo, but prazosin was apparently nontoxic, possibly because of the swift reduction or oxidation of the reactive metabolite to alcohol or acid derivatives that are easily excreted in urine and feces (Erve et al., 2008; Amunom et al., 2011). For furosemide, it is safe in humans at the therapeutic dosage (1–2 mg/kg), whereas toxic in rodents overdosed (>200 mg/kg). Glucuronidation is the primary biotransformation process in vivo, and reduction of metabolic oxidation of the parent drug to the reactive species was found to lower the risk of hepatotoxicity in patients (Perez et al., 1979). These find-

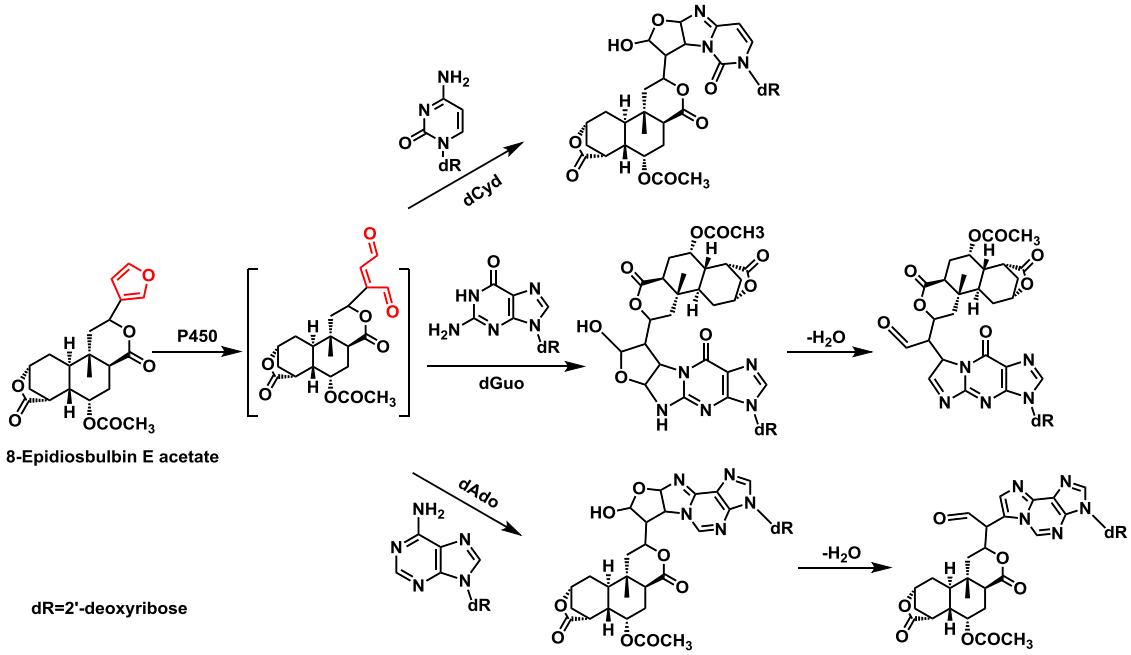


Scheme 11. Proposed pathways of protein and amino acid adduction resulting from the metabolic activation of EEA (Lin et al., 2016b).

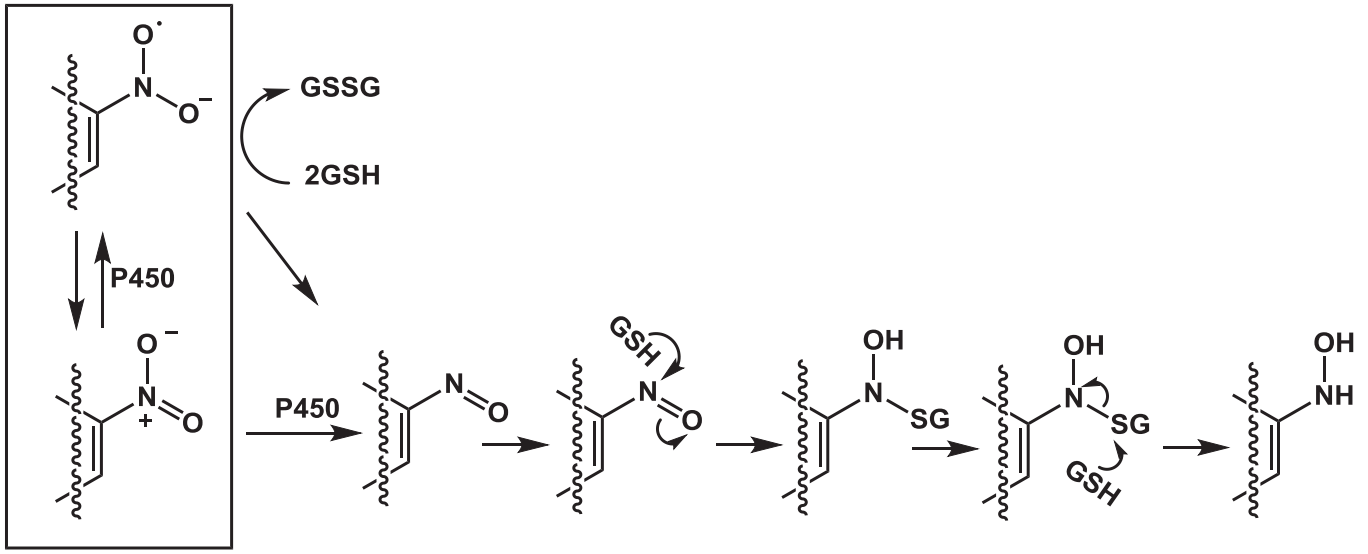
ings remind us that the rapid excretion of chemicals may avoid the adverse reactions induced by metabolic activation. Based on the point, scientists could properly consider introducing polar functional group(s) in the design of new drugs to alter their disposition properties.

Conclusion and Perspectives

Furan-containing compounds have been reported to induce liver and lung injury. Metabolic activation is apparently required for the development of the toxicities. Formation of epoxides and/or *cis*-enedials is the



Scheme 12. Proposed reaction of EEA-derived *cis*-enedial with DNA bases (Lin et al., 2019).



Scheme 13. Proposed mechanisms of P450- and GSH-mediated reduction of nitroaromatics (Li et al., 2019).

initial step for the reported organ toxicities. Numerous studies indicated these reactive intermediates deplete cellular GSH and bind to proteins to generate GSH conjugates and protein adduction. The depletion of GSH could induce oxidative stress, and protein modification could result in protein dysfunction, inducing varieties of toxicities.

Not all furan compounds are toxic, and whether they are toxic depends on their structures. Those furans carrying high electron density often display toxicities, since high density of π-electron facilitates the metabolic oxidation of furan rings to *cis*-enedial reactive metabolites responsible for toxicities. However, those furans with steric hindrance may attenuate the metabolic activation, although π-electron is rich enough for oxidation. Nitro-substituted furans have difficulty being oxidized, due to low density of π-electron. Nitrofurans are often metabolically reduced to nitrosos and hydroxyamines, which execute their toxicities. Additionally, the substituent features may compete the binding toward to the heme for oxidation, and the competition slows down and even blocks the oxidation of furan rings.

Furan-containing compounds exist widely in the globe and are ubiquitous in human life. Approximately 2.2 million have been documented in Scifinder Database, and over 8000 furans have been studied for various reasons. Concerns about human risk to the toxicities of furans have been increasing. So far, only a very small fraction of toxic furans has been identified. Clearly, a mechanism-based platform is needed to screen toxic furans. According to mechanistic understanding, one could speculate the prevention and even interference of the liver injury by slowing down the accumulation of the reactive metabolites.

Authorship Contributions

Participated in research design: Tian, Peng, Zheng.
Wrote or contributed to the writing of the manuscript: Tian, Zheng.

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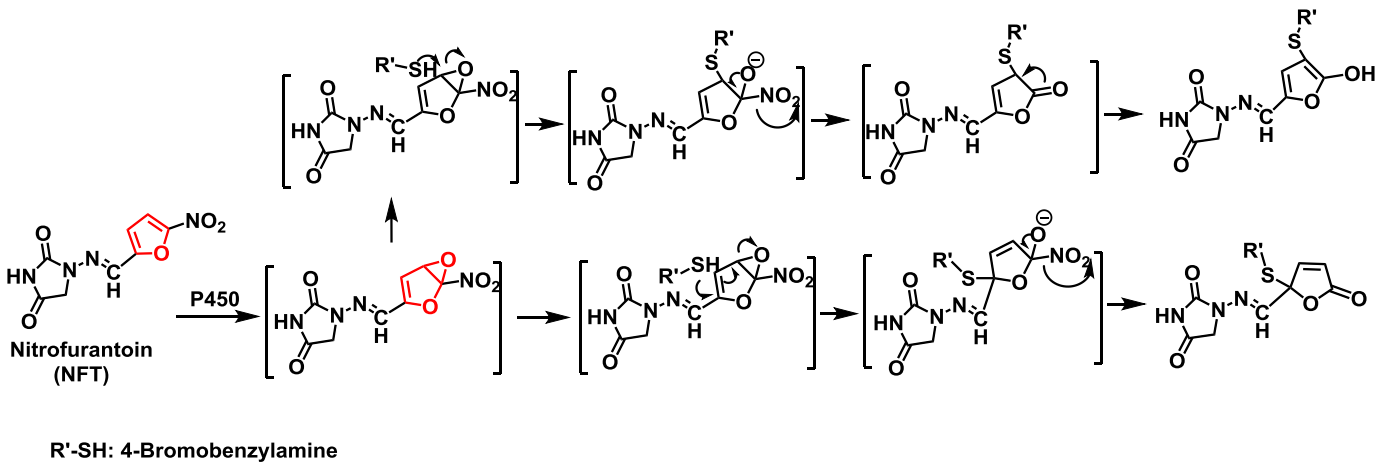
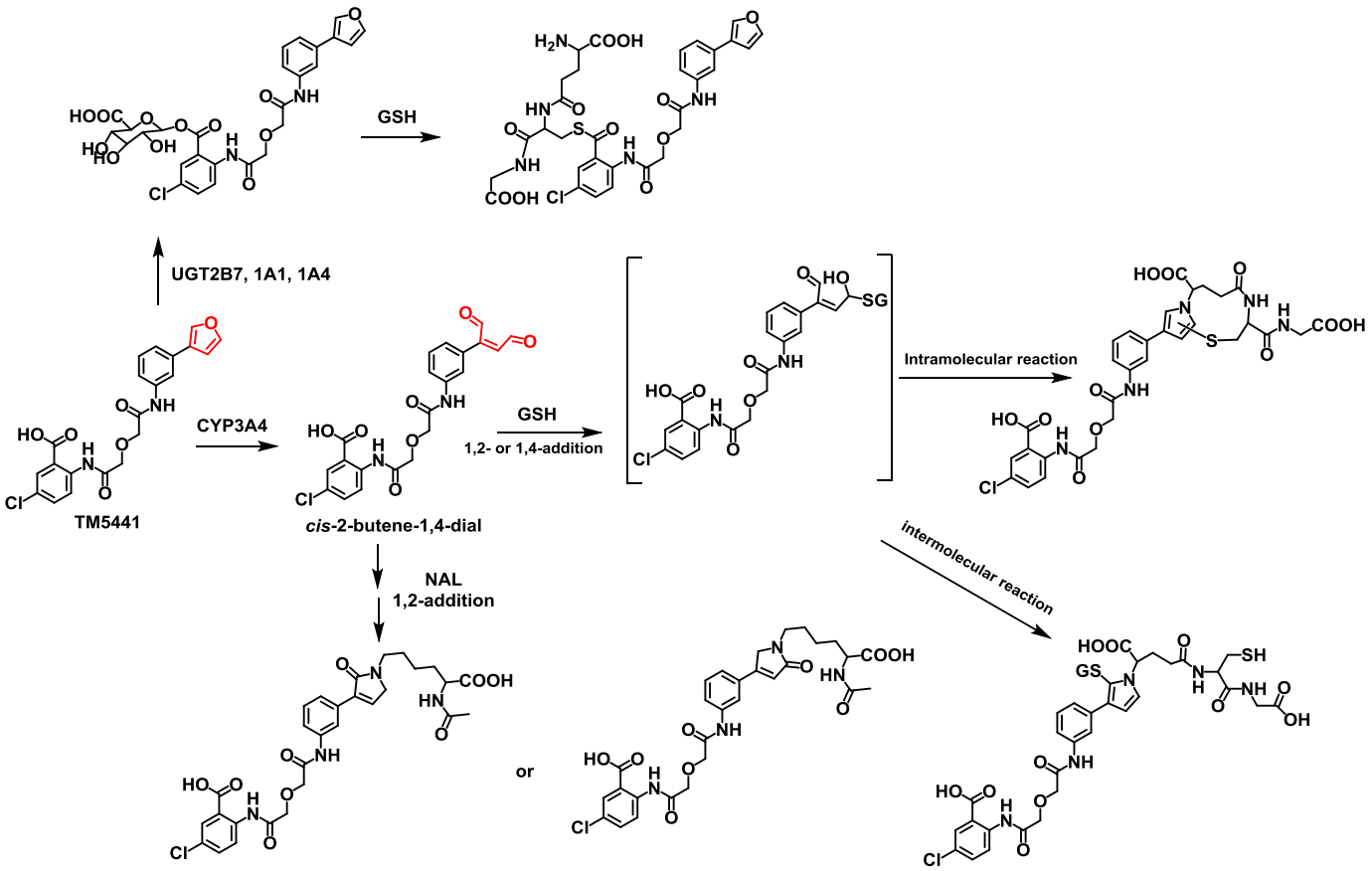


Scheme 14. Proposed pathways for the formation of NFT-derived conjugates by P450-mediated oxidation (Li et al., 2017).



Scheme 15. Metabolic activation of TM5441 mediated by P450 and UDP-glucuronosyltransferases (Lang et al., 2020).

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