Chemical Research in Toxicology

REVIEW

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Structural Alert/Reactive Metabolite Concept as Applied in Medicinal Chemistry to Mitigate the Risk of Idiosyncratic Drug Toxicity: A Perspective Based on the Critical Examination of Trends in the Top 200 Drugs Marketed in the United States

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Supporting Information

ABSTRACT: Because of a preconceived notion that eliminating reactive metabolite (RM) formation with new drug candidates could mitigate the risk of idiosyncratic drug toxicity, the potential for RM formation is routinely examined as part of lead optimization efforts in drug discovery. Likewise, avoidance of "structural alerts" is almost a norm in drug design. However, there is a growing concern that the perceived safety hazards associated with structural alerts and/or RM screening tools as standalone predictors of toxicity risks may be over exaggerated. In addition, the multifactorial nature of idiosyncratic toxicity is now well recognized based upon observations that mechanisms other than RM formation (e.g., mitochondrial toxicity and inhibition of bile salt export pump (BSEP)) also can account for certain target organ toxicities. Hence, fundamental questions arise such as: When is a molecule that contains a structural alert (RM positive or negative) a cause for concern? Could the molecule in its parent form exert toxicity? Can a low dose drug candidate truly mitigate metabolism-dependent and -independent idiosyncratic toxicity risks? In an effort to address these questions, we have retrospectively examined 68 drugs (recalled or associated with a black box warning due to idiosyncratic toxicity) and the top 200 drugs (prescription and sales) in the United States in 2009 for trends in physiochemical characteristics, daily doses, presence of structural alerts, evidence for RM formation as well as toxicity mechanism(s) potentially mediated by parent drugs. Collectively, our analysis revealed that a significant proportion (~78−86%) of drugs associated with toxicity contained structural alerts and evidence indicating that RM formation as a causative factor for toxicity has been presented in 62−69% of these molecules. In several cases, mitochondrial toxicity and BSEP inhibition mediated by parent drugs were also noted as potential causative factors. Most drugs were administered at daily doses exceeding several hundred milligrams. There was no obvious link between idiosyncratic toxicity and physicochemical properties such as molecular weight, lipophilicity, etc. Approximately half of the top 200 drugs for 2009 (prescription and sales) also contained one or more alerts in their chemical architecture, and many were found to be RM-positive. Several instances of BSEP and mitochondrial liabilities were also noted with agents in the top 200 category. However, with relatively few exceptions, the vast majority of these drugs are rarely associated with idiosyncratic toxicity, despite years of patient use. The major differentiating factor appeared to be the daily dose; most of the drugs in the top 200 list are administered at low daily doses. In addition, competing detoxication pathways and/or alternate nonmetabolic clearance routes provided suitable justifications for the safety records of RM-positive drugs in the top 200 category. Thus, while RM elimination may be a useful and pragmatic starting point in mitigating idiosyncratic toxicity risks, our analysis suggests a need for a more integrated screening paradigm for chemical hazard identification in drug discovery. Thus, in addition to a detailed assessment of RM formation potential (in relationship to the overall elimination mechanisms of the compound(s)) for lead compounds, effects on cellular health (e.g., cytotoxicity assays), BSEP inhibition, and mitochondrial toxicity are the recommended suite of assays to characterize compound liabilities. However, the prospective use of such data in compound selection will require further validation of the cellular assays using marketed agents. Until we gain a better understanding of the pathophysiological mechanisms associated with idiosyncratic toxicities, improving pharmacokinetics and intrinsic potency as means of decreasing the dose size and the associated "body burden" of the parent drug and its metabolites will remain an overarching goal in drug discovery.

Received: April 25, 2011
Published: June 24, 2011

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1.0. INTRODUCTION

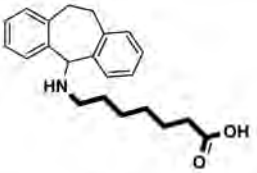
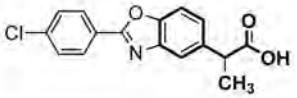
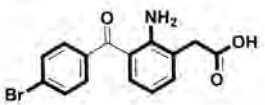
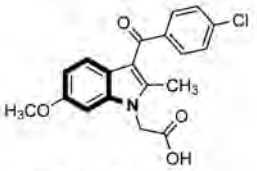
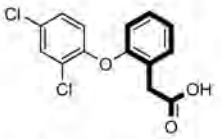
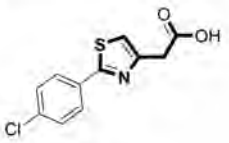
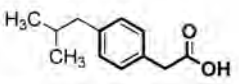
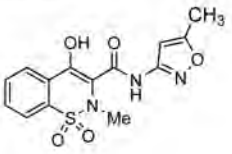
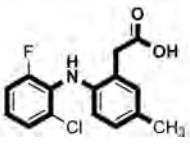
Despite tremendous strides in the field of chemical toxicology, reliably predicting the occurrence of idiosyncratic adverse drug reactions (IADRs) for new drug candidates represents a significant challenge. The term "idiosyncratic" simply represents terminology suggesting that little is known about the underlying mechanism and that the toxicity is unpredictable. Many IADRs possess an immune component and occur in only a subset of patients either acutely or as a delayed response. The observations that certain IADRs (e.g., hypersensitivity associated with the antiretroviral agent abacavir) are linked to specific human leukocyte antigen (HLA) genes provide compelling evidence for the immune-mediated component of these toxicities.[1] IADRs can manifest as a rare and sometimes life-threatening reaction (e.g., hepatotoxicity, skin rashes, and blood dyscracias) in drug-treated patients that cannot be explained based on the primary pharmacology of the drug. For instance, nefazodone is used to treat depression but can cause fatal hepatotoxicity. Idiosyncratic toxicities are, by definition, difficult to reproduce in the human population, and there are few, if any, generally applicable animal models for examining these toxicities.[2] Because the frequency of occurrence in patients is very low, these reactions are often not detected until the drug has gained broad exposure in a large patient population.

The precise mechanisms of IADRs remain unclear; however, it is believed that the majority of these reactions are caused by immunogenic conjugates formed from the reaction of an electrophilic reactive metabolite (RM) of a drug with cellular proteins resulting in direct cellular dysfunction or an immune response via the formation of a hapten. Much of the groundwork of the RM theory was laid by James and Elizabeth Miller who elegantly demonstrated that the carcinogenic and hepatotoxic activity of aminoazo dyes resulted from their metabolic activation (also referred to as bioactivation) to RMs.[3,4] Over the decades, bioactivation of xenobiotics, including drugs, has been clearly implicated in causing DNA modification and mutations relevant to cancer.[5] The extension of the RM concept to idiosyncratic drug toxicity was first provided from studies on the anti-inflammatory agent and hepatotoxicant acetaminophen.[6] Mechanistic studies, which established the cytochrome P450 (CYP) mediated oxidation of acetaminophen to a reactive quinone−imine intermediate,[7] capable of depleting levels of the endogenous antioxidant glutathione (GSH) and/or binding covalently to liver proteins leading to liver injury, have served as a paradigm for drug toxicity assessment over the decades. Studies with model compounds and drugs such as acetaminophen have aided in defining the role that chemical stress and bioactivation can have in biological outcomes that may be triggered by RMs. These include effects on cell signaling/defense, transcription factors, apoptosis, necrosis, and inflammation, activation of the innate and adaptive immune systems.

Under the basic premise that a RM-negative compound could mitigate immune-mediated IADR risks, most drug discovery efforts have implemented screens to assess RM formation from new compounds with the ultimate goal of minimizing or eliminating this liability through iterative design. General avoidance of functional groups that have been associated with RM formation (referred to as "structural alerts")[8] is almost a norm in drug design, particularly at the lead optimization/candidate selection stage. While these approaches appear reasonable in the quest for safer medicines, there is a growing concern that the perceived safety hazards associated with incorporation of a structural alert may be over exaggerated. For instance, almost half of all new small molecule drugs launched in the past five years possess at least one structural alert. Moreover, the vast majority of drugs possess a phenyl ring, which is a structural alert in its own right, since phenyl ring metabolism to the corresponding phenol can proceed through a reactive epoxide intermediate. Aromatic ring epoxidation also represents the rate-limiting step in the carcinogenic and/or hepatotoxic effects of organic solvents such as benzene and bromobenzene. In addition, there are real concerns that simple RM screening tools,[9] such as GSH trapping and covalent binding assays, may be too stringent and could halt the development of useful new medicines. These concerns stem from the fact that several blockbuster drugs contain structural alerts and are subject to bioactivation (RM formation and covalent binding to cellular proteins) but are devoid of idiosyncratic toxicity.[10]

In addition to these challenges, the simplistic notion that the absence of RM formation from a drug candidate serves as a guarantee of its safety is not necessarily true. For example, there is no evidence that the idiosyncratic hepatotoxicity associated with the recalled thrombin inhibitor ximelagatran (**22**) is associated with RM formation,[11] and as such, the drug does not exhibit any alerts in its chemical structure (Table 1). It is also well recognized that IADRs can be multifactorial in nature. Mechanisms other than or in addition to RM formation can individually or collectively account for idiosyncratic liver injury. For instance, hepatotoxicity

Table 1. Structural Alert and RM Analysis for Drugs Withdrawn Due to IADRs

Drug	Indication	Phys. Chem.	M W	Log P	TPSA[a] (Å^2)	Reason for Withdrawal	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[c]
Amineptine (**1**) 	Antidepressant	Acid	337	2.51	49.30	Hepatotoxicity Cutaneous ADRs	200	Short to medium chain fatty acid	Yes[56−58]
Benoxaprofen (**2**) 	NSAID	Acid	301	3.82	58.90	Hepatotoxicity	300-600	Arylacetic acid	Yes[20,28,235]
Bromfenac (**3**) 	NSAID	Acid	334	3.28	80.40	Hepatotoxicity	25–50	Aniline, Arylacetic acid, Bromobenzene	Yes[52]
Clometacin (**4**) 	NSAID	Acid	357	4.46	66.84	Hepatotoxicity	450	Aniline	N.D.[c]
Fenclofenac (**5**) 	NSAID	Acid	297	4.70	46.53	Hepatotoxicity	1200	Arylacetic acid	N.D.[c]
Fenclozic acid (**6**) 	NSAID	Acid	253	2.57	49.66	Hepatotoxicity	300	Heteroarylacetic acid, Thiazole	N.D.
Ibufenac (**7**) 	NSAID	Acid	192	3.37	37.30	Hepatotoxicity	2400	Arylacetic acid	Yes[29,30]
Isoxicam (**8**) 	NSAID	Acid	335	1.61	108.3	Cutaneous ADRs	200	None	N.D.[c]
Lumiracoxib (**9**) 	NSAID	Acid	293	4.65	49.30	Hepatotoxicity	400	Arylacetic acid, *p*-Alkylaniline	Yes[49,50]

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Table 1. Continued

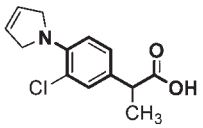
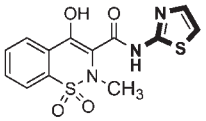
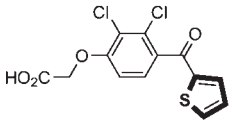
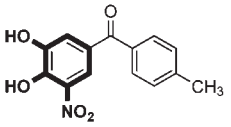
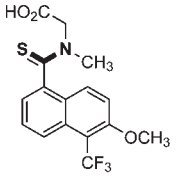
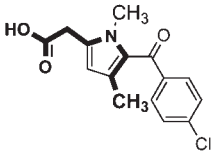
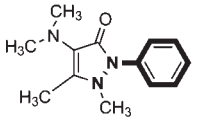
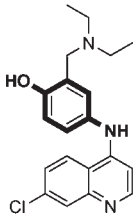
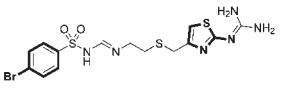
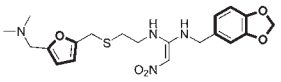
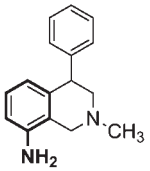
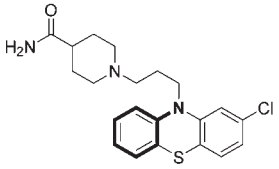
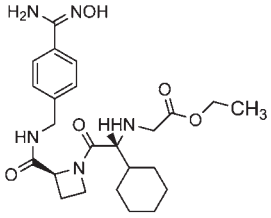
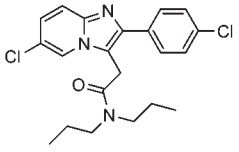
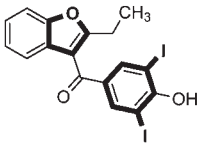
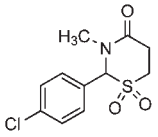
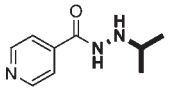
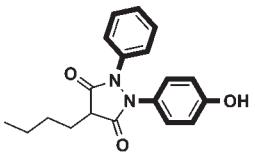
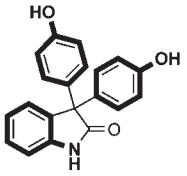
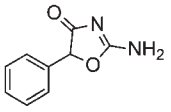
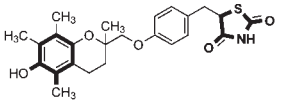
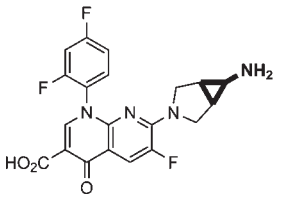
Drug	Indication	Phys. Chem.	M W	Log P	TPSA [a] (Å^2)	Reason for Withdrawal	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[d]
Pirprofen (**10**) 	NSAID	Acid	251	2.60	40.50	Hepatotoxicity	800	Arylacetic acid, Aniline, Olefin	Yes[54]
Sudoxicam (**11**) 	NSAID	Acid	337	1.79	99.10	Hepatotoxicity	50	2-Aminothiazole	Yes[70]
Tienilic acid (**12**) 	Diuretic	Acid	331	3.23	63.60	Hepatotoxicity	250–500	Thiophene	Yes[63–67]
Tolcapone (**13**) 	Anti-Parkinson	Acid	273	3.24	109.34	Hepatotoxicity	300–600	Catechol, Nitrobenzene	Yes[74]
Tolrestat (**14**) 	Antidiabetic	Acid	357	3.43	49.70	Hepatotoxicity	400	Thioamide	N.D.[e]
Zomepirac (**15**) 	NSAID	Acid	291	2.94	57.60	Anaphylaxis	600	Heteroarylacetic acid, Alkylpyrrole	Yes[31,32,47]
Aminopyrine (**16**) 	Analgesic	Base	231	0.57	26.79	Blood dyscrasias	3000	Arylhydrazine	Yes[87]
Amodiaquine (**17**) 	Antimalarial	Base	355	5.46	47.86	Hepatotoxicity Blood dyscrasias	200	*p*-Hydroxyaniline	Yes[80–82]
Ebrotidine (**18**) 	H2 receptor antagonist	Base	477	2.61	135.2	Hepatotoxicity	800	Bromobenzene, 2-Aminothiazole	N.D.[e]

Table 1. Continued

Drug	Indication	Phys. Chem.	M W	Log P	TPSA [a] (Å^2)	Reason for Withdrawal	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[d]
Niperotidine (**19**) 	H2 receptor antagonist	Base	434	2.39	106.8	Hepatotoxicity	460	Furan, 1,3-Benzdioxole	N.D.[e]
Nomifensine (**20**) 	Antidepressant	Base	238	2.37	29.30	Hepatotoxicity Blood dyscrasias	125	Aniline	Yes[85]
Pipamazine (**21**) 	Antiemetic	Base	401	3.91	49.57	Hepatotoxicity	15	Aniline	N.D.[e]
Ximelagatran (**22**) 	Thrombosis	Base	473	1.37	146.35	Hepatotoxicity	48	None	No[11]
Alpidem (**23**) 	Anxiolytic	Neutral	404	5.58	35.91	Hepatotoxicity	25–150	None	Yes[52,90]
Benziodarone (**24**) 	Uricosuric	Neutral	518	6.35	46.50	Hepatotoxicity	300	Furan, Iodobenzene	Yes[f,92]
Chlormezanone (**25**) 	Anxiolytic	Neutral	273	1.57	54.50	Cutaneous ADRs	600	None	N.D.[e]
Iproniazid (**26**) 	Antidepressant	Neutral	179	0.25	53.50	Hepatotoxicity	25–150	Alkylhydrazine	Yes[94]
Oxyphenbutazone (**27**) 	NSAID	Neutral	324	2.71	60.80	Blood dyscrasias	800	Anilide, *p*-Hydroxyaniline, hydrazide	N.D.[e]

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Table 1. Continued

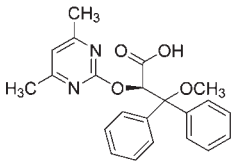
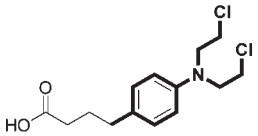
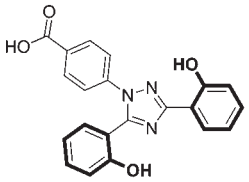
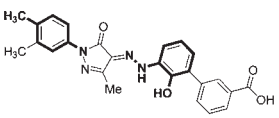
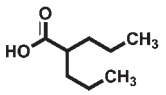
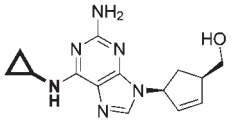
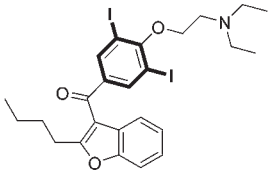
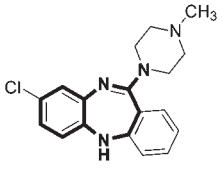
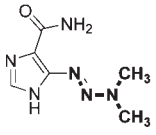
Drug	Indication	Phys. Chem.	M W	Log P	TPSA [a] (Å^2)	Reason for Withdrawal	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[d]
Oxyphenisatine (**28**) 	Laxative	Neutral	317	2.49	69.50	Hepatotoxicity	50	Anilide, Phenol	N.D.[e]
Pemoline (**29**) 	ADHD[c]	Neutral	176	0.46	66.70	Hepatotoxicity	110	None	N.D.[e]
Troglitazone (**30**) 	Antidiabetic	Neutral	441	5.58	84.80	Hepatotoxicity	600	Thiazolidinedione, *o*-Alkylphenol	Yes[99]
Trovafloxacin (**31**) 	Antibacterial	Neutral	416	-1.1	99.20	Hepatotoxicity	100–500	Cyclopropylamine	Yes[105]

[a] TPSA = topological polar surface area. [b] Dose range includes the maximum recommended daily dose. [c] ADHD = attention deficit hyperactivity. [d] Metabolic activation refers to (a) evidence for reactive metabolite formation via characterization of conjugates with nucleophiles and/or (b) demonstration of metabolism-dependent covalent binding to hepatic tissue. [e] N.D. not determined (no published accounts of metabolic activation in the primary literature). [f] Covalent binding in human hepatic tissue demonstrated with benzbromarone (the dibromo version of benziodarone).

associated with the antidiabetic agent troglitazone (**30**) (Table 1) has been attributed to (a) RM formation, (b) inhibition of the ATP-binding cassette transporter bile salt export pump (BSEP) by the parent drug and its sulfate metabolite, and (c) mitochondrial dysfunction and cell death.[12] BSEP is critically involved in the secretion of bile salts into bile; its impairment may lead to cholestasis and accumulation of cytotoxic bile salts in hepatocytes and, consequently, to liver disease.[13] Genetic studies have shown that polymorphism(s) in the gene coding for BSEP and/or inherited mutations lead to progressive familial intrahepatic cholestasis and severe liver disease.[14,15] Mitochondrial dysfunction induced by drugs can originate from several different mechanisms, including the inhibition of fatty acid β-oxidation, the uncoupling of electron transport from ATP synthesis, and the opening of the mitochondrial permeability transition pore leading to irreversible collapse of the transmembrane potential and release of pro-apoptotic factors.[16,17] Overall, the observations with troglitazone imply that both metabolism-dependent and independent mechanisms may need to be considered when truly attempting to de-risk the IADR potential of new drug candidates.

Several excellent reviews in the area of RMs have been published that focus on retrospective structure−toxicity relationships and are mostly aimed at strengthening the bioactivation hypothesis as a causative factor in drug toxicity.[18−21] These papers reaffirm the notion of general avoidance of structural alerts in drug design as a strategy to minimize idiosyncratic toxicity. However, examination of the structural trends with drugs approved over the last five years or so clearly suggests an overlap between chemical drug space and chemical space occupied by molecules possessing structural alerts. Furthermore, some medicinally important functional groups that are classified as structural alerts, such as the aniline moiety, can be quite challenging to mimic by isosteric replacement. Hence, questions arise such as, when is a molecule that contains a structural alert (RM positive or negative) a cause for concern? Are some structural alerts more promiscuous than others? Could the molecule in its parent form exert toxicity? Can a low dose drug candidate truly mitigate metabolism-dependent and -independent IADR risks? In an effort to address these difficult questions and hopefully provide answers to some of them, we embarked on the present venture wherein we retrospectively examined 68 drugs (recalled or associated with a black box warning (BBW) due to idiosyncratic toxicity in the US or European markets) for trends in physicochemical characteristics, the presence of structural alerts, evidence for RM formation, and protein covalent binding as well as toxicity mechanism(s) potentially mediated through cellular effects by the parent drug.

Table 2. Structural Alert and RM Analysis for Drugs Associated with a Black Box Warning (BBW) Due to IADRs

Drug	Indication	Phys. Chem.	M W	Log P	TPSA[a] (Å^2)	Reason for BBW	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[e]
Ambrisentan (**32**) 	Pulmonary hypertension	Acid	378	3.75	80.48	Hepatotoxicity	10	None	N.D.[f]
Chlorambucil (**33**) 	Anticancer	Acid	304	3.63	40.54	Blood dyscrasias Bone marrow suppression	12	Nitrogen mustard, Alkyl halide, *p*-Alkylaniline	Yes[111]
Deferasirox (**34**) 	Iron chelator	Acid	373	3.66	105.72	Hepatotoxicity	1200	Phenol	N.D.[f]
Eltrombopag (**35**) 	Thrombocytopenia	Acid	442	5.24	114.60	Hepatotoxicity	75	*o*-Hydroxyaniline, *p*-Alkylaniline, Hydrazine	Yes[112]
Valproic acid (**36**) 	Anticonvulsant	Acid	144	2.76	37.30	Hepatotoxicity	1000–2400	Short to medium chain carboxylic acid	Yes[117–120]
Abacavir (**37**) 	Antiretroviral	Base	286	0.80	98.60	Hepatotoxicity	600	Cyclopropylamine	Yes[121]
Amiodarone (**38**) 	Antiarrhythmic	Base	645	8.94	38.70	Hepatotoxicity	1600	Iodobenzene	N.D.[f]
Clozapine (**39**) 	Antipsychotic	Base	326	3.71	30.90	Blood dyscrasias	300–900	Benzodiazepine	Yes[127–131]
Dacarbazine (**40**) 	Anticancer	Base	182	0.48	95.40	Hepatotoxicity	140–315	Dialkyltriazine	Yes[132]

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Table 2. Continued

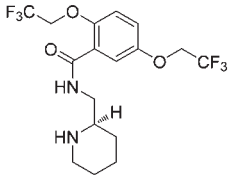
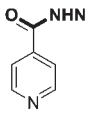
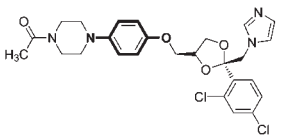
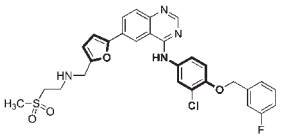
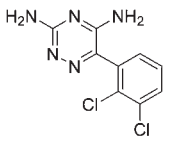
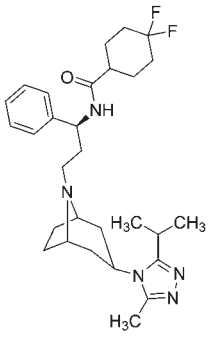
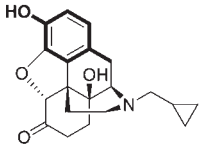
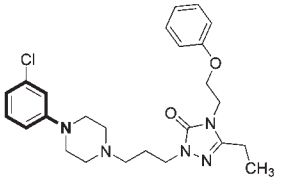
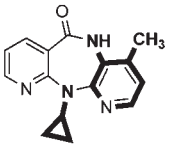
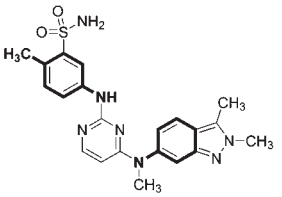
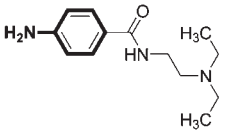
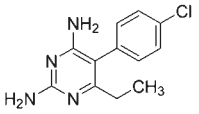
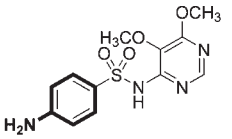
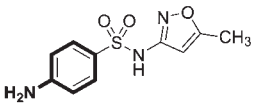
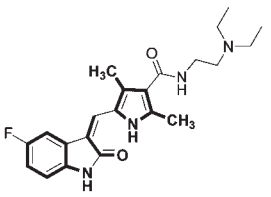
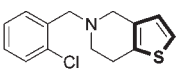
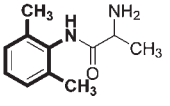
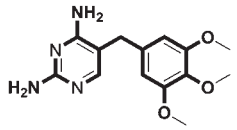
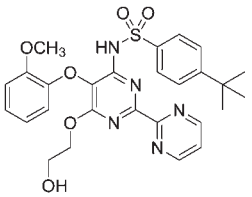
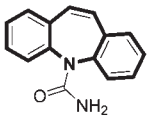
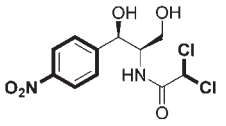
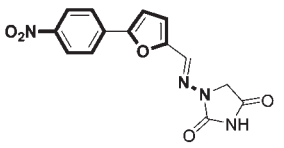
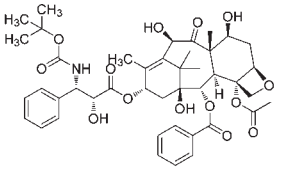
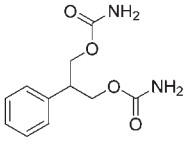
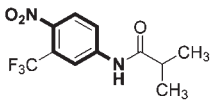
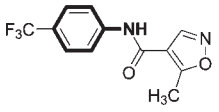
Drug	Indication	Phys. Chem.	M W	Log P	TPSA[a] (Å^2)	Reason for BBW	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[e]
Flecainide (**41**) 	Antiarrhythmic	Base	414	5.81	59.60	Hepatotoxicity	300	None	N.D.[f]
Isoniazid (**42**) 	Antibacterial	Base	137	-0.67	67.50	Hepatotoxicity	300	Hydrazide	Yes[92,136,137]
Ketoconazole (**43**) 	Antifungal	Base	531	3.63	66.84	Hepatotoxicity	400	*p*-Alkoxyaniline	Yes[143]
Lapatinib (**44**) 	Anticancer	Base	581	5.96	101.4	Hepatotoxicity	1500	Furan, *p*-Alkoxyaniline	Yes[146]
Lamotrigine (**45**) 	Anticonvulsant	Base	256	2.53	89.10	Cutaneous ADRs	600	None	Yes[150,151]
Maraviroc (**46**) 	Antiretroviral	Base	514	3.26	60.30	Hepatotoxicity	1200	None	N.D.[f]
Naltrexone (**47**) 	Analgesic	Base	341	0.36	70.00	Hepatotoxicity	50	*p*-Alkylphenol	Yes[156]
Nefazodone (**48**) 	Antidepressant	Base	470	5.72	51.60	Hepatotoxicity	200–600	Aniline	Yes[157,158]

Table 2. Continued

Drug	Indication	Phys. Chem.	M W	Log P	TPSA[a] (Å^2)	Reason for BBW	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[c]
Nevirapine (**49**) 	Antiretroviral	Base	266	2.64	57.10	HepatotoxicityCutaneous ADRs	200	Cyclopropylamine, *o*-Alkylanilide	Yes[165−167]
Pazopanib (**50**) 	Anticancer	Base	437	3.65	115.7	Hepatotoxicity	800	*p*-Alkylaniline, Aniline	N.D.[f]
Procainamide (**51**) 	Antiarrhythmic	Base	235	1.42	58.40	Blood dyscrasias	1000	Aniline	Yes[168,169]
Pyrimethamine[c] (**52**) 	Antiprotozoal	Base	248	3.00	76.70	Cutaneous ADRs	75	None	N.D.[f]
Sulfadoxine[c] (**53**) 	Antibacterial	Base	310	1.23	115.4	Cutaneous ADRs	1500	Aniline	N.D.[f]
Sulfamethoxazole (**54**) 	Antibacterial	Base	253	0.56	93.78	Blood dyscrasias Bone marrow toxicity Hepatotoxicity Cutaneous ADRs	2400	Aniline	Yes[170−172]
Sunitinib (**55**) 	Anticancer	Base	370	4.53	73.50	Hepatotoxicity	50	Anilide, Michael acceptor, Alkylpyrrole	N.D.[f]
Ticlopidine (**56**) 	Antithrombotic	Base	263	4.39	31.5	Blood dyscrasias	500	Thiophene	Yes[180−182]
Tocainide (**57**) 	Antiarrhythmic	Base	192	0.26	55.10	Blood dyscrasias	1800	Anilide, *o*-Alkylanilide	N.D.[f]

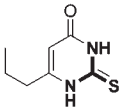
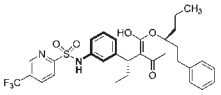
dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Table 2. Continued

Drug	Indication	Phys. Chem.	M W	Log P	TPSA[a] (Å^2)	Reason for BBW	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[c]
Trimethoprim (**58**) 	Antibacterial	Base	290	0.98	104.45	Blood dyscrasia Bone marrow toxicity Hepatotoxicity Cutaneous ADRs	200	*o*- and *p*-Alkylaniline, *p*-Alkylaromatic ether, Dialkoxyaromatic	Yes[176,177]
Bosentan (**59**) 	Pulmonary Hypertension	Neutral	551	4.16	143.5	Hepatotoxicity	250	None	N.D.[f]
Carbamazepine (**60**) 	Anticonvulsant	Neutral	236	2.38	46.33	Blood dyscrasias	1600	Styrene, Aniline	Yes[190−193]
Chloramphenicol (**61**) 	Antibacterial	Neutral	322	1.28	121.4	Blood Dyscrasias	6000	Nitrobenzene, Dihaloalkane	Yes[199,201]
Dantrolene (**62**) 	Muscle relaxant	Neutral	314	1.63	122.8	Hepatotoxicity	400	Furan, Nitrobenzene, Hydrazine	Yes[204]
Docetaxel (**63**) 	Anticancer	Neutral	808	4.08	224	Hepatotoxicity Cutaneous ADRs Blood dyscrasias	-----[d]	None	N.D.[f]
Felbamate (**64**) 	Anticonvulsant	Neutral	238	0.5	104.6	Hepatotoxicity Blood dyscrasias	3600	None	Yes[208−210]
Flutamide (**65**) 	Antiandrogen Anticancer	Neutral	276	3.33	80.91	Hepatotoxicity	750	Anilide, Nitrobenzene	Yes[219−223]
Leflunomide (**66**) 	Anti-inflammatory	Neutral	270	2.32	50.60	Hepatotoxicity	20	Anilide	Not formed[225]

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Table 2. Continued

Drug	Indication	Phys. Chem.	M W	Log P	TPSA[a] (Å^2)	Reason for BBW	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation[e]
Propylthiouracil (**67**) 	Antithyroid	Neutral	170	0.97	41.13	Blood dyscrasias Hepatotoxicity	1200	Thiourea	Yes[227,228]
Tipranavir (**68**) 	Antiretroviral	Neutral	602	7.54	105.1	Hepatotoxicity	1000	Anilide	N.D.[f]

[a] TPSA = topological polar surface area. [b] Top end of the daily dose is the maximum recommended dose. [c] Marketed as a combination. [d] Intravenous use. [e] Metabolic activation refers to (a) evidence for reactive metabolite formation via characterization of conjugates with nucleophiles and/or (b) demonstration of metabolism-dependent covalent binding to hepatic tissue. [f] N.D., not determined (no published accounts in the primary literature).

The exercise was then repeated for the top 200 drugs (by prescription number and by sales) in the United States in 2009. Trends in the daily dosing regimen of all the drugs were also examined in this analysis. For the overall exercise, it was assumed that adequate safety was demonstrated in regulatory preclinical toxicity studies for each drug; hence, this review only focuses on toxicity of an idiosyncratic nature. The listing for the top 200 drugs was obtained from www.pharmacytimes.com. The database and literature searches conducted in this review were broad in scope and included PubMed (U.S. National Library of Medicine), Scifinder (CAS database), RxList (the Internet drug index for prescription drugs), and the Physicians Desk Reference; it is, however, possible that some information may have been overlooked.

2.0. PHYSICOCHEMICAL TRENDS IN DRUGS ASSOCIATED WITH IADRS

Analysis of the physicochemical attributes of the 68 small molecule drugs listed in Table 1 (drugs withdrawn due to IADRs) and Table 2 (marketed drugs with BBW label due to IADRs) revealed a slight bias toward basic amines (19 carboxylic acids, 29 bases, and 20 neutral compounds). The drugs span a broad range of molecular weights (MW) (137−808 Da), log *P* values (−0.67 to +6.35), and topological polar surface areas (TPSA) (27−224 Å^2), which is consistent with the diversity of their pharmacologic targets. It is interesting to note that a prior study[22] on preclinical compounds found a correlation between compounds possessing high log *P* and low TPSA and an increased likelihood of toxic outcomes in preclinical studies, which does not necessarily hold true with idiosyncratic toxicities. Despite the diversity in physicochemical characteristics and chemical structures, it is interesting to note that a frequent cause for recalls and/or the BBW label is hepatotoxicity. Blood dyscracias (characterized by aplastic anemia, agranulocytosis, or hemolytic anemia), cutaneous ADRs (serious manifestations include Stevens−Johnson syndrome, and toxic epidermal necrolysis), and anaphylaxis are other notable IADRs leading to drug withdrawal and/or restriction in use. Although IADRs are often considered unrelated to dose, the drugs depicted in the two Tables present a different

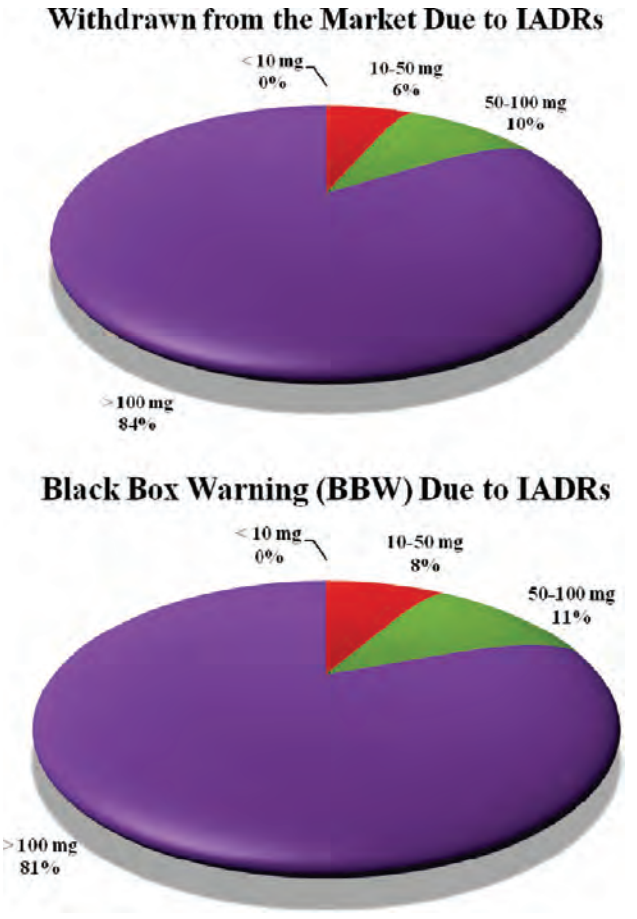


Figure 1. Maximum recommended daily dose assessments for drugs associated with idiosyncratic toxicity.

view. With relatively few exceptions (e.g., pipamazine (**21**) and oxyphenisatine (**28**) (Table 1), ambrisentan (**32**), chlorambucil (**33**), and leflunomide (**66**) (Table 2)) the vast majority of drugs associated with toxicity are high to very high dose drugs

(100−2400 mg) (Figure 1). As such, this empirical observation makes no assumptions pertaining to mechanism/structure, biochemical reactivity, etc. but indicates that toxicity is most likely related to body burden of the drug and/or its metabolite(s).

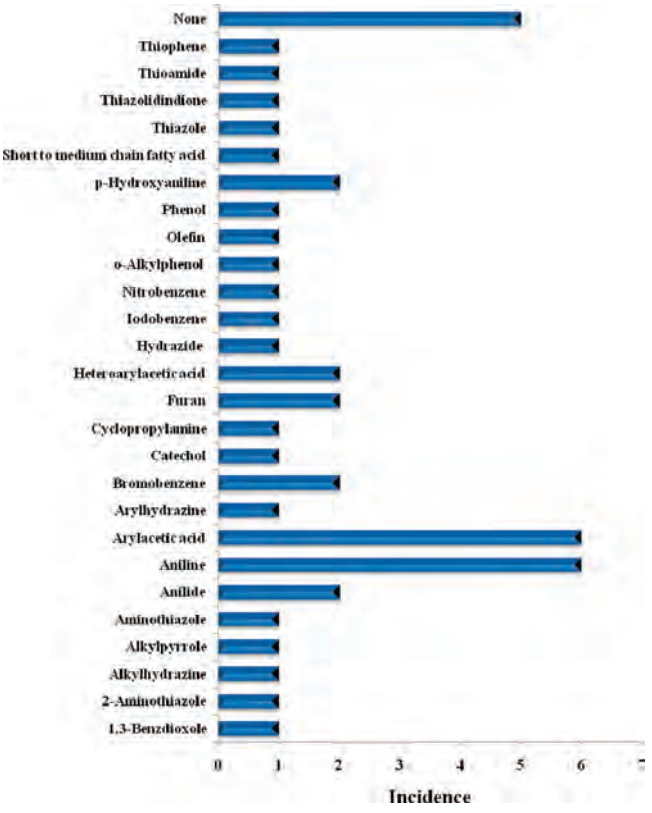


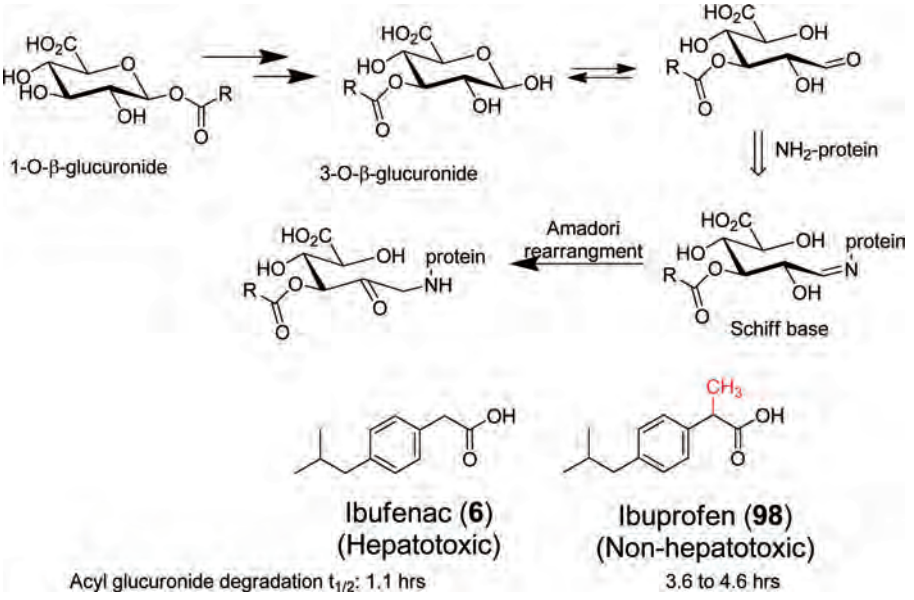
Figure 2. Structural alert analysis in drugs withdrawn due to IADRs.

3.0. STRUCTURAL ALERT AND RM ANALYSES FOR DRUGS RECALLED DUE TO IADRS

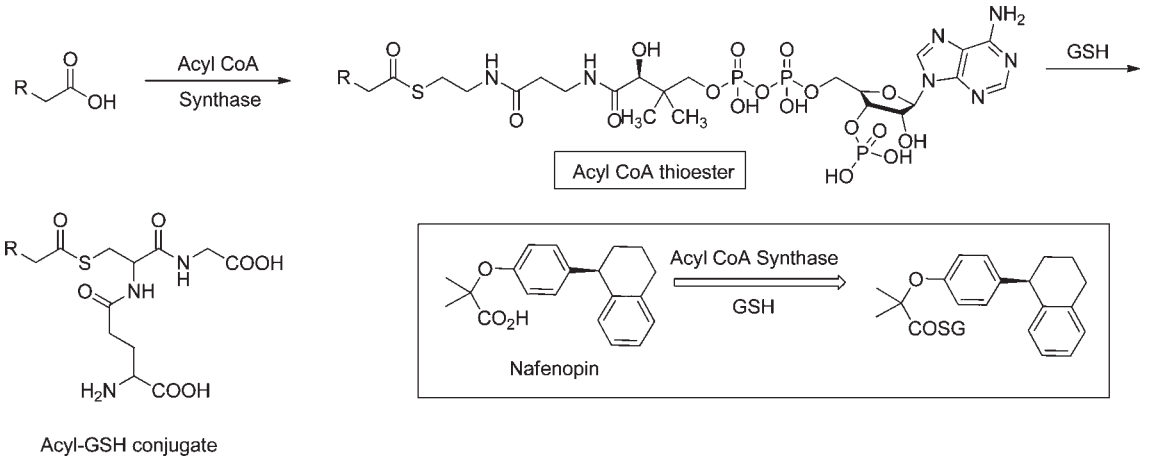
Structural alerts as outlined in recent reviews[23,24] were identified through a manual inspection of the chemical structures. The phenyl ring was not considered as a structural alert in this analysis; however, phenols, alkoxyaromatic ethers/amines, and alkylaromatic ethers/amines were flagged as alerts because of the greater likelihood of sequential metabolism to quinonoid species (e.g., quinone-methides, quinone-imines, and imine-methides). Of the 31 withdrawn drugs shown in Table 1, 26 (84%) possess one or more structural alerts (highlighted in bold) capable of forming RM(s).[23,24] With respect to reoccurring structural alert types, substituents known to form electrophilic acyl glucuronides (e.g., arylacetic acids) and anilines/anilides appear frequently in the drugs (Figure 2). Of the 26 drugs that feature structural alerts in their chemical architecture, 18 (69%) have been shown to undergo metabolic activation as defined by RM formation (characterization of adducts with biological nucleophiles such as GSH) and/or covalent binding to target organ tissue (e.g., liver microsomes). Out of the 31 drugs listed in Table 1, evidence for additional potential toxicity liabilities such as BSEP inhibition and/or mitochondrial dysfunction has been presented for five drugs.

3.1. Acidic Compounds. Most acidic drugs that have been removed from the market belong to the arylacetic acid (e.g., bromfenac (**3**), ibufenac (**7**), zomepirac (**15**), etc.), propionic acid (benoxaprofen (**2**), pirprofen (**10**), etc.), and enol-carboxamide (e.g., isoxicam (**8**) and sudoxicam (**11**)) class of nonsteroidal anti-inflammatory drugs (NSAIDs). With the exception of isoxicam that is associated with cutaneous ADRs, all other NSAIDs have been withdrawn due to idiosyncratic hepatotoxicity. Despite this dubious distinction for frequent recalls, more than 20 generic acidic NSAIDs are still available either in prescription form (e.g., diflunisal) or over-the-counter (e.g., aspirin, ibuprofen, naproxen, etc.) treatment options for rheumatoid arthritis. In contrast to benoxaprofen-induced idiosyncratic

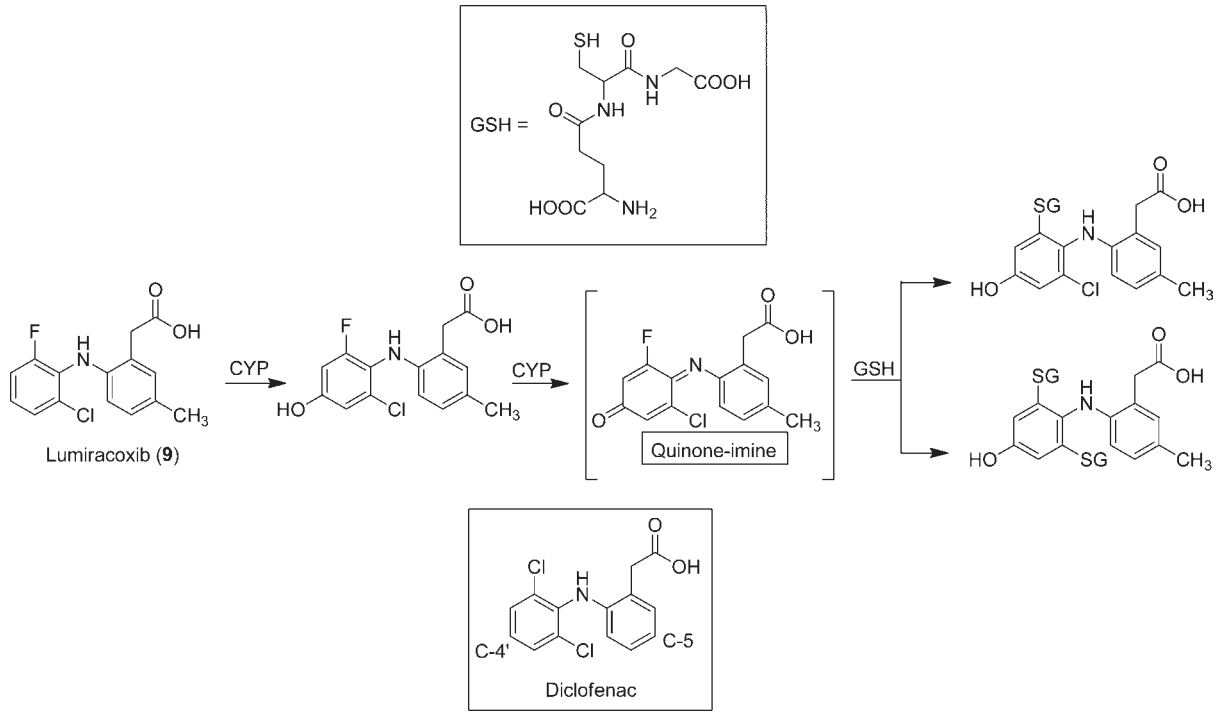
Scheme 1. Mechanism of Acyl Glucuronide Adduction to Proteins: The Story of the Magic Methyl-hepatotoxin Ibufenac (6) versus Non-Hepatotoxin Ibuprofen (98)



Scheme 2. Acyl CoA Thioester Metabolites of Carboxylic Acids As Electrophiles



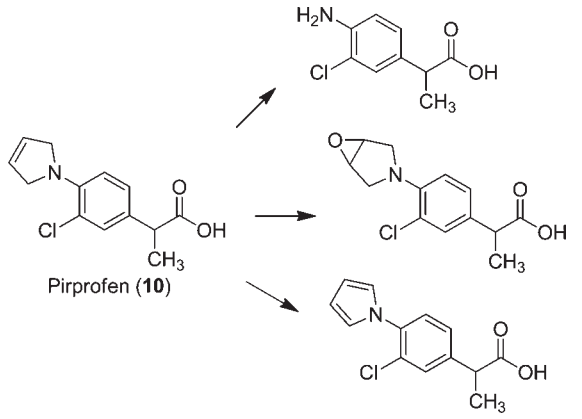
Scheme 3. Metabolic Activation of Lumiracoxib (9) by CYP Enzymes in a Manner Similar to the Hepatotoxic NSAID Diclofenac



hepatotoxicity, which involves biliary cirrhosis and cholestasis,[25] many IADRs associated with recalled carboxylic acid-based NSAIDs are believed to be immune-mediated and, for the most part, have been linked to the corresponding β-1-*O*-acyl glucuronide (also referred to as acyl glucuronide) derivatives, which constitute the major metabolites of many NSAIDs in humans. Acyl glucuronides can covalently modify proteins via a simple transacylation reaction or through an acyl migration within the β-*O*-glucuronide unit to a reactive α-hydroxy-aldehyde intermediate.[26−32] Condensation between the α-hydroxy-aldehyde motif and a lysine residue or an amine group of the *N* terminus in proteins leads to the formation of a glycated conjugate. The formation of the iminium species is reversible but may be followed by an Amadori rearrangement of the imino sugar to a more chemically stable 1-amino-2-keto product (Scheme 1). Structure−toxicity correlations between acyl glucuronide migration rates to the Schiff base and covalent binding to liver microsomes and serum albumin have revealed that carboxylic acids with a higher degree of alkyl substitution at the α-carbon exhibit lower reactivity with protein nucleophiles suggesting that inherent electronic and steric effects likely modulate the overall rate of acyl glucuronide rearrangement.[33−35] A rather dramatic illustration of this effect is evident upon a comparison of the hepatotoxicant ibufenac (**6**) and ibuprofen (**98**, also refer to Table 1 in Supporting Information) (Scheme 1), two of the safest over-the-counter NSAIDs on the market. The daily doses are comparable (200−400 mg), and the only structural difference is the presence of the α-methyl substituent in ibuprofen. While both drugs are extensively glucuronidated in

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Scheme 4. Oxidative Metabolism of Pirprofen (10) in Humans



animals and humans, the presence of the extra α-methyl substituent in ibuprofen slows acyl glucuronide formation/migration relative to ibufenac, thus providing a possible explanation for the toxicological differences. However, the notion of acyl glucuronide reactivity as a general predictor of IADR has been challenged based upon clinical covalent binding data with ibuprofen and related NSAIDs such as naproxen (**159**, see Table 1 in Supporting Information) with impeccable safety records.[36] For instance, protein adducts of ibuprofen, generally considered to be a safe NSAID, have been detected in human plasma and appear to derive from the acyl glucuronide upon the basis of a strong correlation between the extent of covalent binding and the plasma area under the curve (AUC) of ibuprofen acyl glucuronide.[29]

In addition to acyl glucuronide formation, NSAIDs can also be bioactivated to electrophilic acyl-coenzyme A thioester derivatives (acyl-CoAs) (Scheme 2). The biochemical basis for acyl-CoA thioester formation with carboxylic acids is well documented in lipid metabolism, where fatty acids are metabolized by acyl-CoA ligases to acyl-CoAs before entrance into mitochondria.[37] Acyl-CoA metabolites of several NSAIDs including zomepirac (**15**) have been detected *in vitro* and *in vivo*.[38−40] Acyl-CoA derivatives of carboxylic acid drugs act as intermediates in a number of phase II conjugation reactions, including amino acid conjugation. As such, the intermediate acyl CoA metabolites of carboxylic acids are thioester derivatives, which possess sufficient electrophilicity toward nucleophilic reactions with amino acid residues on proteins as well as with GSH (Scheme 2).[41−46] For instance, the hypolipidemic drug nafenopin (see Scheme 2) was shown to transacylate liver proteins following *in vitro* incubations with liver homogenates, resulting in amide and thioester linkages with protein lysine and cysteine amino acid residues, respectively, and to linearly correlate the plasma AUC of nafenopin protein acylation with the AUC of nafenopin-CoA formation.[46] As was noted with acyl glucuronides, the substitution pattern around the acyl CoA metabolites greatly influences chemical reactivity; increasing substitution at the α-carbon generally correlates with a decrease in reactivity with nucleophiles.[43]

Cytochrome P450 (CYP)-mediated bioactivation to RMs also occurs with some carboxylic acid-based NSAIDs. For instance, in addition to acyl glucuronidation,[31,32] the pyrrole ring of zomepirac (**15**) undergoes oxidative metabolism to a reactive epoxide intermediate that can be trapped by GSH.[47] Likewise, lumiracoxib (**9**) is an arylacetic acid derivative whose metabolism in

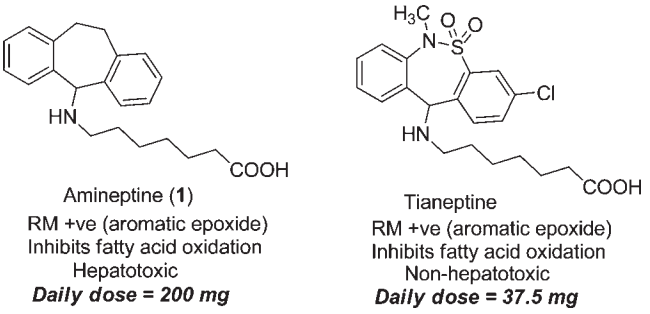


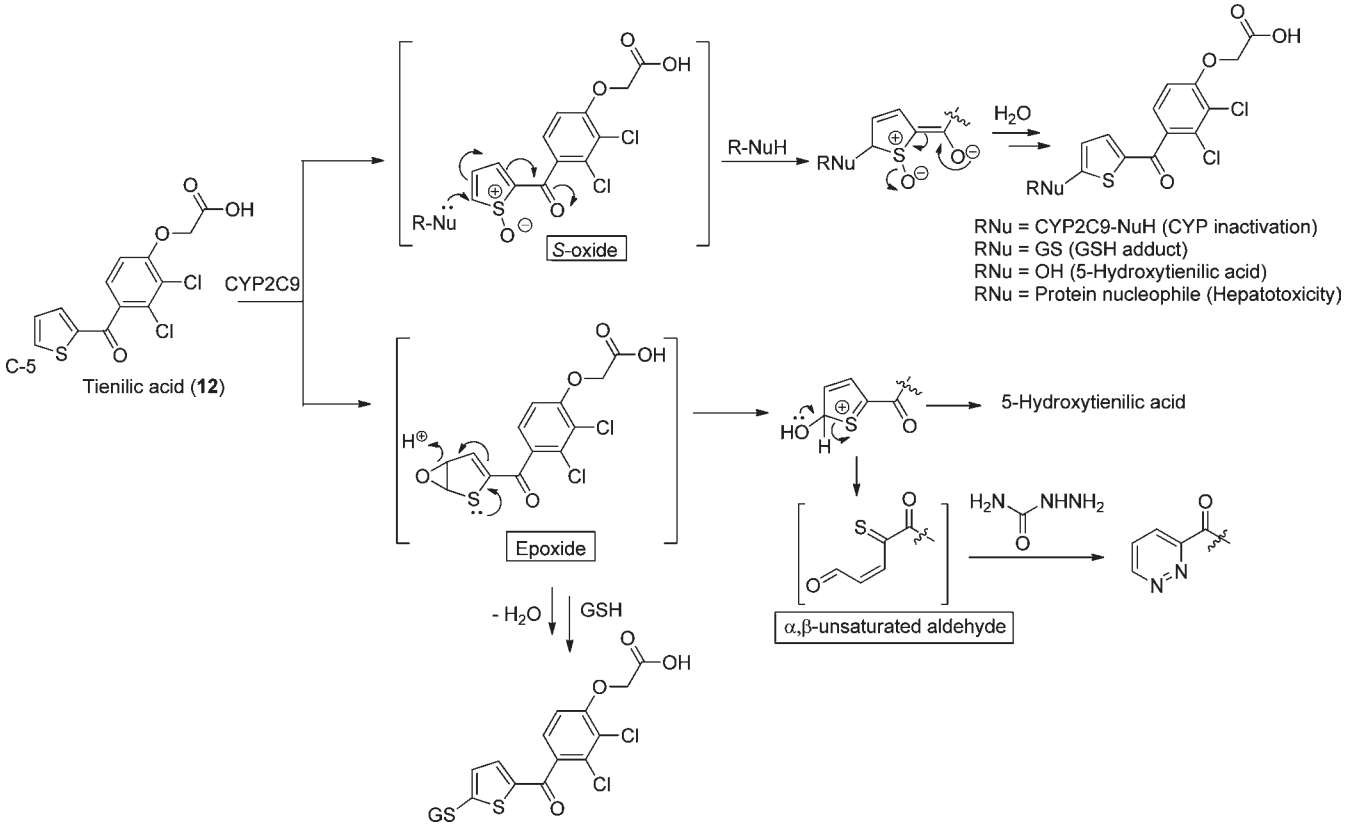
Figure 3. Structure−toxicity relationship between hepatotoxic amineptine (**1**) and nonhepatotoxic tianeptine: influence of dose size.

humans is mostly catalyzed by CYP enzymes.[48] 4′-Hydroxylumiracoxib, the major circulating metabolite of lumiracoxib in humans, is oxidized to a reactive quinone-imine intermediate in human liver microsomes (HLM) that can be trapped by GSH (Scheme 3).[49,50] Lumiracoxib is structurally related to diclofenac, a drug itself known to induce a rare but severe hepatotoxicity in exposed patients. Diclofenac undergoes CYP-catalyzed hydroxylation at the 4′- and 5-positions, the products of which are also oxidized to reactive quinone-imine intermediates and characterized as their corresponding thiol adducts in humans (see Scheme 3).[51]

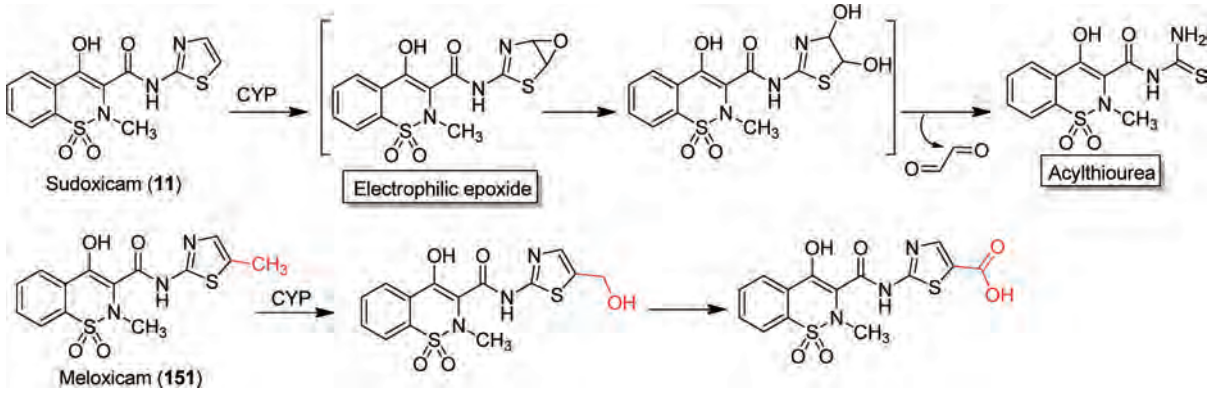
The structure−toxicity relationship for bromfenac (**3**) is complex since mechanistic studies pertaining to RM characterization have not been described. Bromfenac possesses arylacetic acid, aniline, and bromobenzene motifs that can undergo bioactivation through conjugation and/or oxidative processes.[24] Indirect evidence for RM formation through oxidative metabolism can be inferred from the observation that covalent binding to HLM by [^{14}C]-bromfenac occurs in a NADPH-dependent fashion implying a role for CYP enzymes.[52] Similarly, aniline and olefin alerts are also present in the structure of the propionic acid NSAID pirprofen (**10**). In humans, the metabolic pathways of pirprofen involve acyl glucuronidation, olefin epoxidation, aromatization of the pyrroline ring, and heterocyclic ring scission to the aniline metabolite (Scheme 4).[53] In HLM, the oxidative metabolism of pirprofen results in protein- and GSH-reactive metabolites providing evidence for RM formation and thereby a circumstantial link between metabolism and toxicity. The oxidative pathways of pirprofen that lead to RM formation remain to be elucidated.[54] Besides the potential role of RM(s) in toxicity, inhibitory effects on mitochondrial β-oxidation of fatty acids[54] and microsomal triglyceride transfer protein[55] have been noted with the parent NSAID which can also account for the microvesicular steatosis that accompanies liver cell necrosis in humans.

NADPH-dependent microsomal covalent binding is also observed with the tricyclic antidepressant and hepatotoxicant amineptine (**1**) suggestive of RM formation by CYP enzymes.[56−58] The RM is believed to be an epoxide since addition of an epoxide hydrolase inhibitor increases microsomal covalent binding.[56−58] In addition, amineptine is primarily metabolized in humans through β-oxidation on the heptanoic side chain (leading to the corresponding pentanoic and propionic acid metabolites)[59] and is a potent inhibitor of mitochondrial β-oxidation of fatty acids leading to microvesicular steatosis.[60] In contrast to amineptine, the structural analogue tianeptine (Figure 3) appears to be relatively safe in the clinic despite sharing amineptine's liabilities involving bioactivation by CYP into a reactive arene oxide and inhibitory effects on mitochondrial β-oxidation of fatty acids.[60] It is likely

Scheme 5. Bioactivation of the Thiophene Ring in Tienilic Acid (12) to RMs



Scheme 6. Differences in the Metabolic Pathways of Hepatotoxin Sudoxicam (11) and Non-Hepatotoxin Meloxicam (151) in Humans

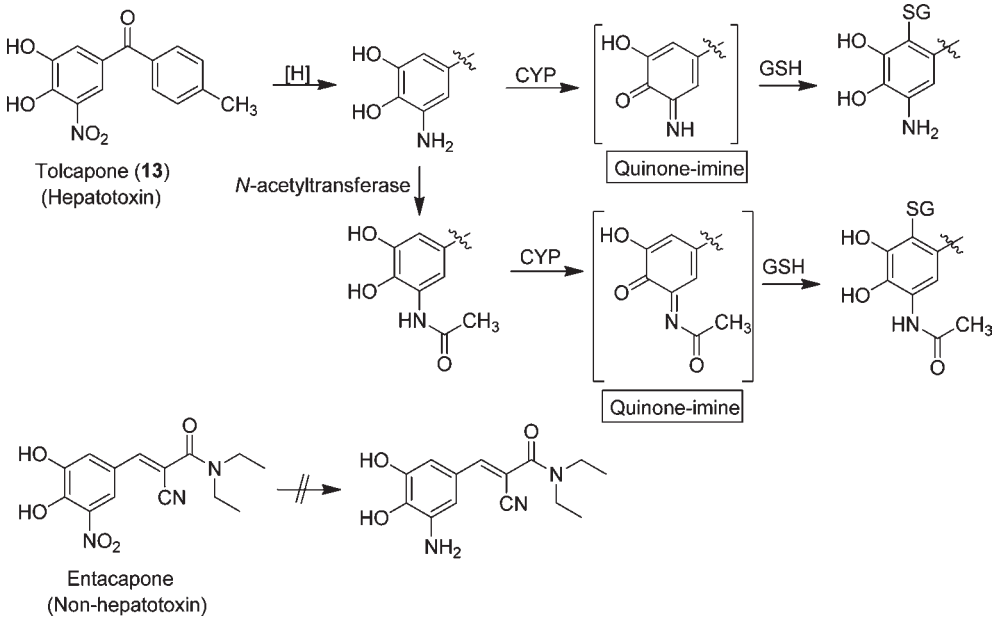


that the improved tolerance of tianeptine in the clinic arises from the ~5−6-fold lower recommended dose relative to that of amineptine. For clometacin (**4**), fenclofenac (**5**), fenclozic acid (**6**), and tolrestat (**14**), there is no information in the literature regarding metabolic activation to RM(s). Fenclofenac is an arylacetic acid derivative, and it is possible that its acyl glucuronide metabolite in humans[61] is responsible for the observed toxicity. With clometacin, the biochemical mechanisms leading to IADRs are not as clear. In one study, toxicity mediated via an immunogenic mechanism was proposed upon the basis of the cytotoxicity findings in human hepatocytes exposed to serum from patients with clometacin-induced hepatitis.[62]

CYP2C9-catalyzed bioactivation of the thiophene ring in tienilic acid (**12**) has been linked to the hepatotoxic effects of this diuretic. In humans, the major urinary metabolite is derived from the hydroxylation at the C-5 position on the thiophene ring, a process that also leads to the formation of protein-reactive metabolites capable of irreversibly inactivating CYP2C9.[63−66] *In vivo* evidence for CYP2C9 inactivation stems from the detection of highly specific anti-LKM2 autoantibodies (known

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Scheme 7. Metabolism of the COMT Inhibitor Tolcapone (13) to RMs[a]



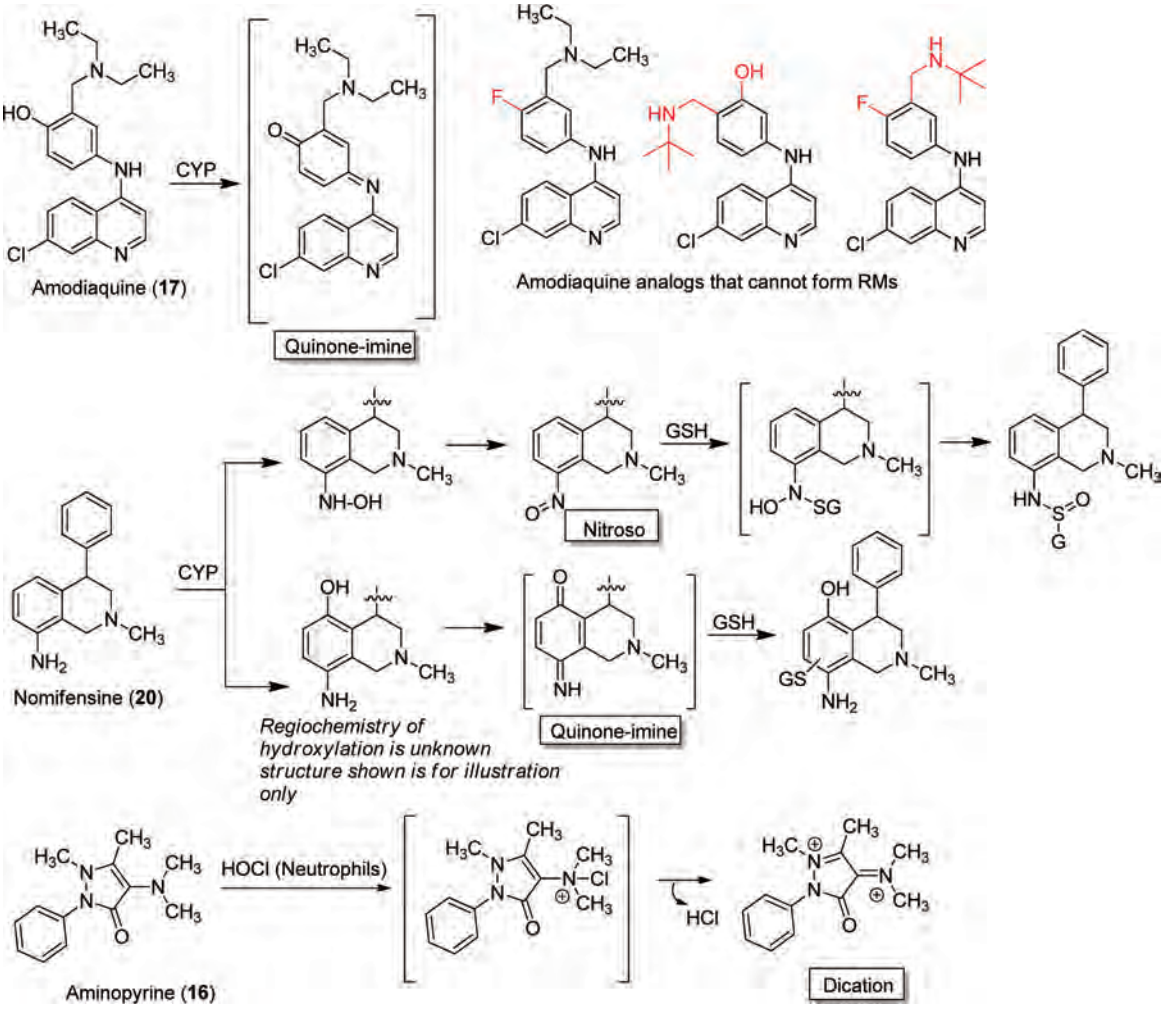
[a] Rationalization for the improved safety of structural analogue entacapone through metabolic differences.

to specifically recognize CYP2C9) in patients affected with tienilic acid-induced hepatitis.[67] Plausible RMs derived from thiophene ring oxidation in tienilic acid include the electrophilic *S*-oxide, which can react at the C-5 position with water to afford the stable 5-hydroxytienilic acid metabolite or with biological nucleophiles (e.g., GSH or amino acid residue in the CYP2C9 active site) (Scheme 5). An alternate pathway for RM formation involving the formation of an electrophilic thiophene ring epoxide followed by ring scission to a γ-thioketo-α,β-unsaturated aldehyde intermediate has also been proposed on the basis of metabolism studies with model thiophene derivatives (see Scheme 5).[68,69]

The hepatotoxicity associated with the enol-carboxamide NSAID sudoxicam (**11**) that led to its suspension from further testing has been attributed to a CYP-catalyzed thiazole ring scission to the acylthiourea metabolite (Scheme 6), which upon hydrolysis would afford the thiourea metabolite, capable of oxidizing GSH and proteins.[70] The bioactivation pathway leading to the acylthiourea also represents the major metabolic fate of sudoxicam in animals and humans.[70] The structurally related NSAID meloxicam (**151**), which makes the top 200 drug list on the basis of dispensed prescriptions (see Table 1 in Supporting Information), does not possess the hepatotoxic liability associated with sudoxicam despite administration at comparable doses. Although introduction of a methyl group at the C-5 position on the thiazole ring in meloxicam is the only structural difference, the change dramatically alters the metabolic profile. Oxidation of the C-5 methyl group to the alcohol (and carboxylic acid) metabolite(s) constitutes the major metabolic fate of meloxicam in humans (Scheme 6).[60] It is noteworthy to point out that the C-5 methyl group on the aminothiazole ring in meloxicam was essential for selective cyclooxygenase (COX)-2 inhibitory potency and was not specifically introduced to eliminate RM formation.[71] Against this backdrop, the biochemical basis for cutaneous ADRs noted with the enol-carboxamide derivative isoxicam (**8**) remains unknown. Isoxicam does not contain a structural alert, and the principal metabolic fate of this drug in humans is innocuous in nature involving CYP-mediated hydroxylation on the methylisoxazole group.[72]

Since its entry onto the market in 1998, the catechol-*O*-methyl transferase (COMT) inhibitor and anti-Parkinson's agent tolcapone (**13**) has been associated with numerous cases of hepatotoxicity, including three cases of fatal fulminant hepatic failure. Although catechol glucuronidation is the major metabolic fate of tolcapone in humans, the corresponding reduction products of the nitrocatechol group, namely, the aniline and *N*-acetylaniline derivatives, have been detected as minor metabolites in human excreta.[73] The two metabolites undergo a facile two-electron oxidation to an electrophilic quinone-imine species that can be trapped by GSH in HLM (Scheme 7).[74] A close association between hepatotoxicity and mutations in the uridine diphospho glucuronosyltransferase (UGT) 1A9 gene (which encodes the UGT isozyme responsible for tolcapone glucuronidation) has been observed in tolcapone-treated patients, suggesting that RM formation through the redox bioactivation pathway may be more pronounced in UGT1A9 poor metabolizer genotypes.[75] Whether risk of tolcapone toxicity is reduced in UGT1A9 extensive metabolizers is not known. From a structural standpoint, entacapone (Scheme 7) also possesses the nitrocatechol moiety, and despite administration at a high daily dose of 1600 mg, the drug is not associated with the IADRs observed with tolcapone. There are several possible reasons that could potentially account for the toxicity differences. First and foremost, metabolic profiling of entacapone in humans indicates that the analogous aniline and *N*-acetylaniline metabolites are not formed; primary metabolic routes in humans involve isomerization of the double bond to the *cis*-isomer followed by catechol glucuronidation, and hydroxylation on the *N*-diethyl substituent.[76] In addition to metabolic differences, tolcapone, but not entacapone, is a potent uncoupler of oxidative phosphorylation and an inhibitor of mitochondrial respiration, actions that in parallel with RM formation can provide an additive effect to the hepatotoxic outcome with tolcapone.[77]

Scheme 8. RM Formation Pathways for Basic Amine Drugs, Amodiaquine (17), Nomifensine (20), and Aminopyrine (16), Which Have Been Withdrawn Due to IADRs

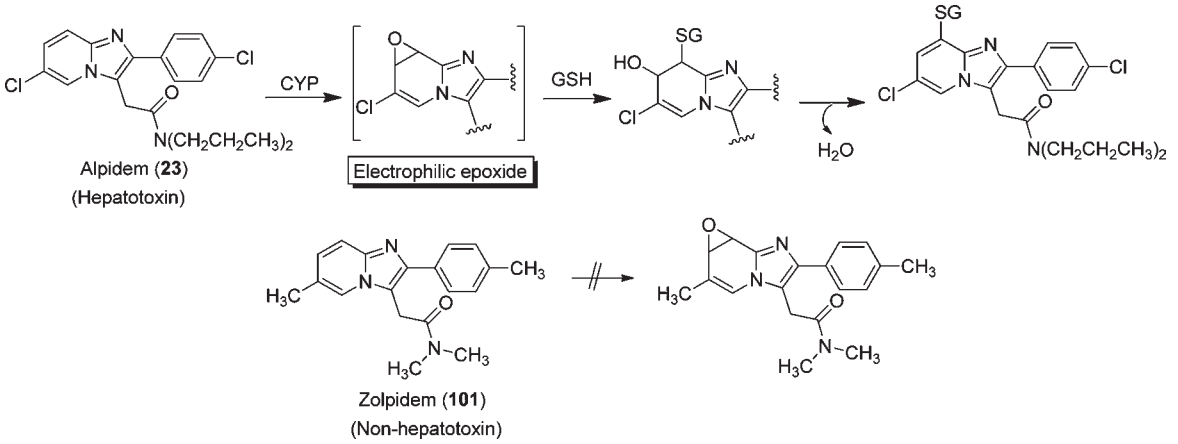


3.2. Basic Compounds. Amodiaquine (**17**) has been widely used for the treatment of malaria. The incidence of fatal ADRs (hepatotoxicity and blood dyscrasias) in patients taking the drug for prophylactic use has resulted in amodiaquine no longer being a frontline therapy for malaria. The detection of IgG antibodies in patients exposed to amodiaquine is consistent with an immune-mediated IADR.[78] The immune-mediated toxicity is believed to originate from the CYP and myeloperoxidase (MPO)-mediated metabolism of the 4-hydroxyaniline motif in amodiaquine and its *N*-deethylated metabolite[79] to reactive quinone-imine species, which can covalently bind to cellular proteins or GSH (Scheme 8).[80,81] Exchanging the *C*-4′-phenolic OH group with a fluorine abolishes RM formation.[82] An alternate approach to prevent RM formation involves the interchange of the 3′- and 4′-substituents in amodiaquine leading to analogues incapable of forming the quinone-imine species (Scheme 8). As such, these strategies have been used to identify back-up clinical candidates that maintain the pharmacologic and disposition profile of amodiaquine but are devoid of RM liability.[83]

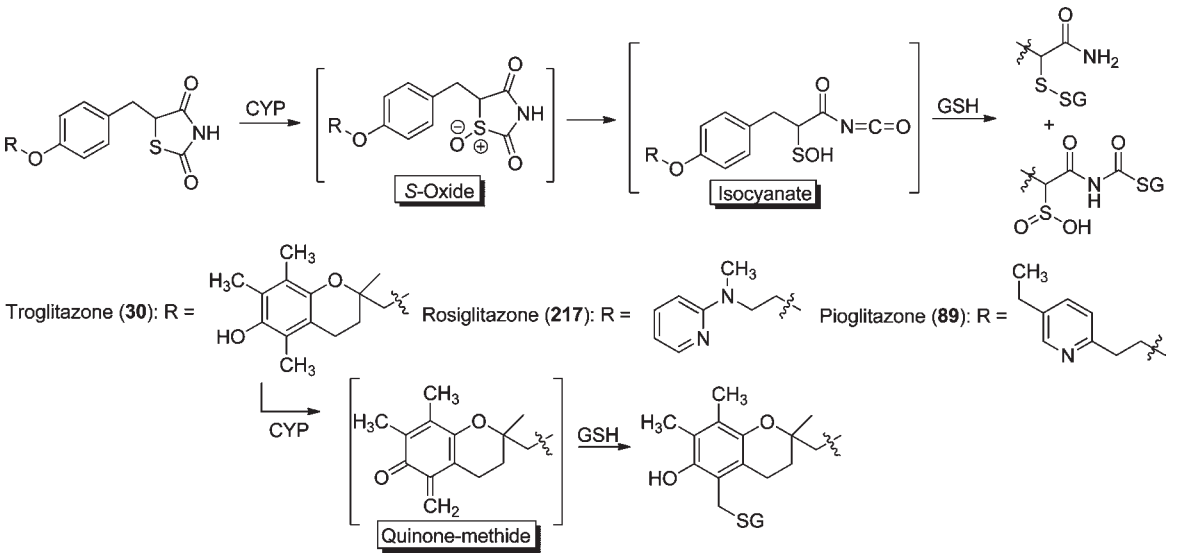
IADRs associated with nomifensine (**20**) have also been linked to its metabolism, the basis for which is the detection of auto-antibodies directed against nomifensine and/or its metabolite(s) in patients who developed immune-mediated blood dyscracias.[84] Oxidative metabolism of the aniline motif in nomifensine by CYP enzymes results in the formation of two distinct RMs (quinone-imine and nitroso intermediates) that can be trapped by GSH (Scheme 8).[85] It is possible that these RMs can also covalently modify proteins leading to hepatotoxicity. The analgesic aminopyrine (**16**) was one of the first drugs to be associated with the induction of blood dyscrasias (agranulocytosis) in humans.[86] Uetrecht et al.[87] have characterized the oxidation of aminopyrine by neutrophil-derived hypochlorous acid (generated as a byproduct of MPO) to a reactive dication metabolite (Scheme 8) that could be responsible for aminopyrine-induced blood dyscrasias.

While structural alerts are present in the hepatotoxicant ebrotidine (**18**), niperotidine (**19**), and pipamazine (**21**), there is no evidence for RM formation with these drugs. Likewise, with ximelagatran (**22**), there is no *in vitro* and/or *in vivo* evidence suggestive of RM formation. Ximelagatran, the first orally active thrombin inhibitor, was recently withdrawn from commercial use due to several cases of hepatotoxicity.[11] Ximelagatran does not contain any structural alerts, and its *in vivo* biotransformation in humans consists of apparently innocuous pathways involving ester hydrolysis and reduction of the amidoxime motif to afford

Scheme 9. Structure−Toxicity Relationship Analysis between the Anxiolytic Agents, Hepatotoxin Alpidem (23) and Non-Hepatotoxin Zolpidem (101)



Scheme 10. Bioactivation of Thiazolidinedione-Containing Anti-Diabetic Drugs in HLM

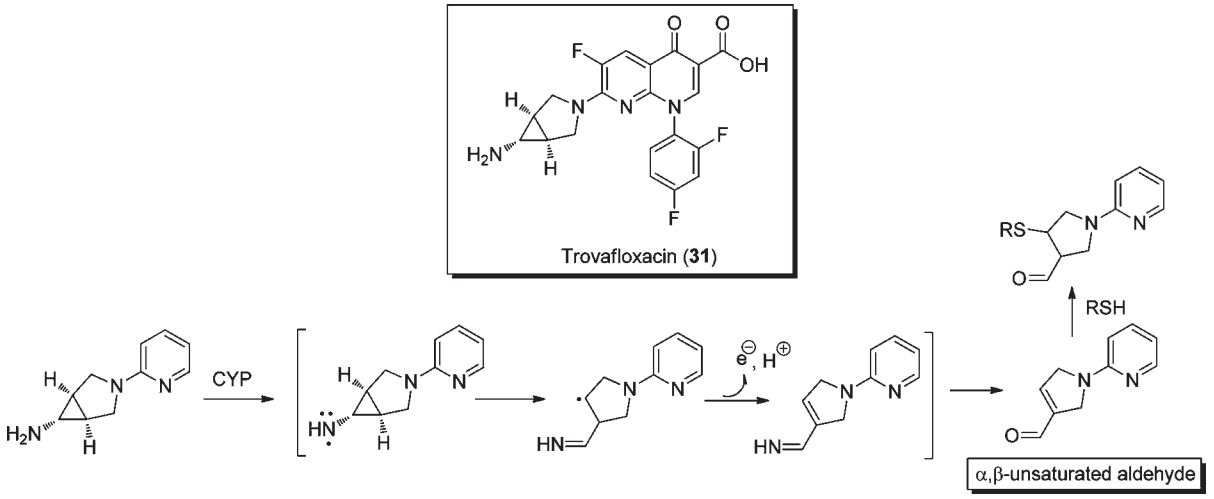


the active ingredient melagatran. However, genome-wide association studies in humans found a strong genetic predisposition to liver injury in patients that carried the major histocompatibility complex alleles DRB1(*)07 and DQA1(*)02, suggesting an immune-based pathogenesis for hepatotoxicity.[88]

3.3. Neutral Compounds. Out of the nine neutral drugs listed in Table 1, there is no literature evidence on RM formation for four of them (chlormezanone (**25**), oxyphenbutazone (**27**), oxyphenisatine (**28**), and pemoline (**29**)). Furthermore, there are no alerts in the structures of chlormezanone and pemoline. Alpidem (**23**) was withdrawn from commercial use within the first year of its introduction due to several cases of severe hepatotoxicity.[89] Although alpidem is devoid of a prototypic structural alert, evidence has been presented for CYP-mediated bioactivation of the chloro-imidazopyridine ring to an electrophilic epoxide that reacts with GSH or microsomal protein.[52,90] The detection of mercapturic acid conjugates in human excreta provides evidence for the existence of this pathway *in vivo* (Scheme 9).[90] Interestingly, the structural analogue of alpidem, namely, zolpidem (**101**), that is a member of the top 200 drug list (by prescriptions and sales) is not hepatotoxic. A key structural difference between the two drugs is the replacement of the two chlorine atoms in alpidem with two methyl groups in zolpidem (Scheme 9). Zolpidem is metabolized via the oxidation of both methyl groups to the corresponding alcohol and carboxylic acid metabolites and is not subject to RM formation.[90] In addition to the RM liability, alpidem exhibits potent inhibition of mitochondrial respiration and depletes GSH in primary hepatocyte cultures, phenomena that are not observed with zolpidem even at high concentrations.[91] Direct evidence for RM formation with benziodarone (**24**) is not available; however, NADPH-dependent covalent binding to HLM and human hepatocytes has been demonstrated with the corresponding brominated analogue and uricosoric agent benzbromarone, suggesting the formation of RMs.[92] In addition, benzbromarone also has additional liabilities such as potent BSEP inhibition *in vitro* and is a mitochondrial inhibitor that causes necrosis and induction of apoptosis in isolated rat hepatocytes.[93]

The high incidence of hepatocellular injury associated with the clinical use of the antidepressant iproniazid (**26**) has been linked to its metabolism involving amide bond hydrolysis to

Scheme 11. Insights into RM Formation with Trovafloxacin (31) via the Use of a Model Compound Containing the Cyclopropylamine Functionality



isopropylhydrazine, a potent hepatotoxicant in rats.[94] The hepatotoxic effects of iproniazid parallels CYP-mediated oxidation of isopropylhydrazine to a RM that binds covalently to microsomal protein.[95] The release of propane as a byproduct of iproniazid metabolism suggests that isopropylhydrazine is oxidized to an intermediate that generates a protein-reactive propyl radical.[96] In fact, radicals have been detected in the microsomal oxidation of hydrazines, and nitrogen evolution has been recorded.[97] The observation that human antimitochondria autoantibodies appearing in iproniazid-induced immunoallergic hepatitis recognize human liver monoamine oxidase B is consistent with the irreversible inactivation of the flavoprotein via oxidation of the hydrazine motif in the drug.[98] Whether this process plays a role in the immune-mediated hepatotoxicity is unclear.

Both the chromane and the thiazolidinedione ring systems of troglitazone (**30**) are known to form RMs (quinone-methide and thiazolidinedione ring scission products) by CYP3A4 (Scheme 10).[99] As mentioned earlier, troglitazone and the corresponding sulfate metabolite (the major metabolite of troglitazone in humans) are potent inhibitors of BSEP, which may account for the cholestatic mechanism associated with troglitazone liver injury.[100,101] Furthermore, troglitazone is cytotoxic and also induces mitochondrial dysfunction and cell death in human hepatocytes.[102] Interestingly, the idiosyncratic hepatotoxicity with troglitazone has not been discerned with rosiglitazone (**217**) (Table 2, Supporting Information) and pioglitazone (**89**) (Table 4), which made the top 200 list of drugs either by prescription and/or sales. Rosiglitazone and pioglitazone contain the thiazolidinedione scaffold, which is metabolized to yield RMs similar to those detected with troglitazone (Scheme 10).[103] While rosiglitazone and pioglitazone do not lead to mitochondrial dysfunction,[102] they do inhibit BSEP at concentrations comparable to that of troglitazone.[93] While oxidation of the thiazolidenedione ring and/or BSEP inhibition is a common theme in these drugs, a key difference lies in their daily doses, troglitazone (200–400 mg/day) versus rosiglitazone and pioglitazone (<10 mg/day), suggesting that a threshold for safety can be developed even with potent inhibitors of BSEP based on overall daily dose. It should also be noted that in rats troglitazone but not rosiglitazone or pioglitazone was a potent antagonist to bile acid-mediated farnesoid X receptor activation.[104] This activity could result in a decrease in bile acid secretion and increase in bile acid synthesis, leading to hepatotoxicity via the accumulation of bile acids.

The broad-spectrum antibiotic trovafloxacin (**31**) has been associated with several cases of acute liver failure and/or liver-injury related fatalities. This rare but serious hepatotoxicity led to the withdrawal of trovafloxacin from the market in many countries. Interestingly, trovafloxacin is the only drug in the fluoroquinolone class of antibiotics (e.g., see Table 1 in Supporting Information for the structures of levofloxacin (**109**) and ciprofloxacin (**157**), which are in the list of the most prescribed drugs) whose use has been severely restricted due to an acceptable risk of idiosyncratic hepatotoxicity. From a structural standpoint, trovafloxacin contains the cyclopropylamine functionality at the C-7 position of the fluoroquinolone scaffold (Scheme 11), a feature absent in other fluoroquinolone-based antibiotics. Studies with a model cyclopropylamine derivative have revealed cyclopropylamine ring bioactivation by CYPs and MPO to a reactive α,β-unsaturated aldehyde that is trapped with GSH (Scheme 11).[105] Microarray analysis has also revealed substantial gene expression changes following treatment of human hepatocytes with trovafloxacin compared to those of other marketed fluoroquinolones.[106] The expression profile induced by trovafloxacin is markedly distinct from that of other fluoroquinolones in that genes involved in oxidative stress are upregulated consistently by trovafloxacin. In HepG2 cells, trovafloxacin also induces oxidative stress and depletes intracellular GSH levels to a greater extent than other fluoroquinolones.[107] Depletion of GSH appears to fit with the RM theory. Finally, in lipopolysaccharide-pretreated rats administered trovafloxacin, accumulation of neutrophils (PMN cells) in liver tissue has been observed.[108] Prior depletion of PMNs in the animals attenuated liver injury, thus confirming the role of PMN in the hepatotoxicity induced by lipopolysaccharide/trovafloxacin.

4.0. STRUCTURAL ALERT AND RM ANALYSES FOR DRUGS WITH BBW

Of the 37 drugs associated with the BBW listed in Table 2, 29 (78%) contain one or more alerts (highlighted in bold) upon

manual inspection of the chemical structures. Of the 29 drugs that were flagged for alerts, 18 (62%) are known to undergo bioactivation to RMs. A prominent structural alert in many of these drugs is the electron rich aniline motif and derivatives thereof that can participate in the formation of quinonoid species (Figure 4). Interestingly, felbamate does not possess a structural alert but is known to form reactive species. Likewise, the dichlorophenyl ring in lamotrigine, which was not included as a structural alert in this analysis, is bioactivated by CYP enzymes

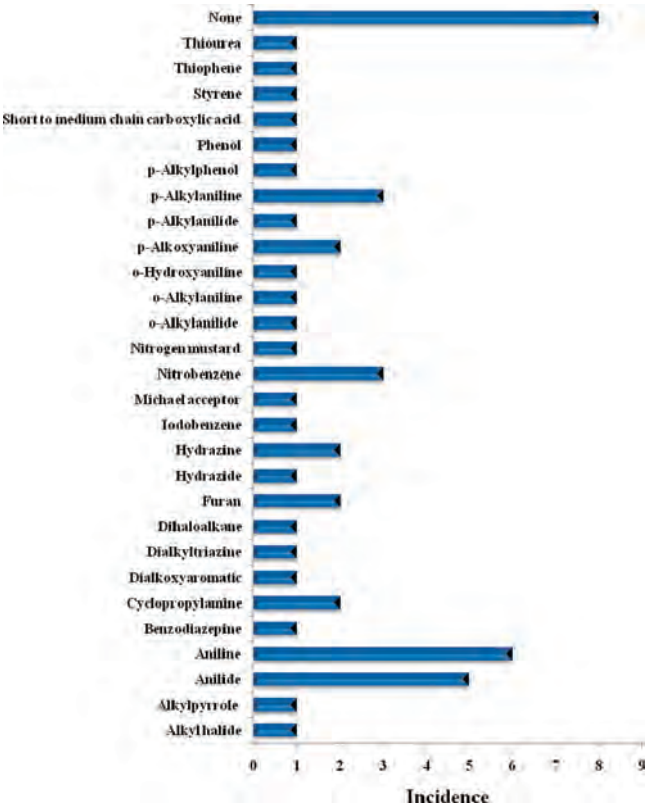


Figure 4. Structural alert analysis in drugs associated with BBW for IADRs.

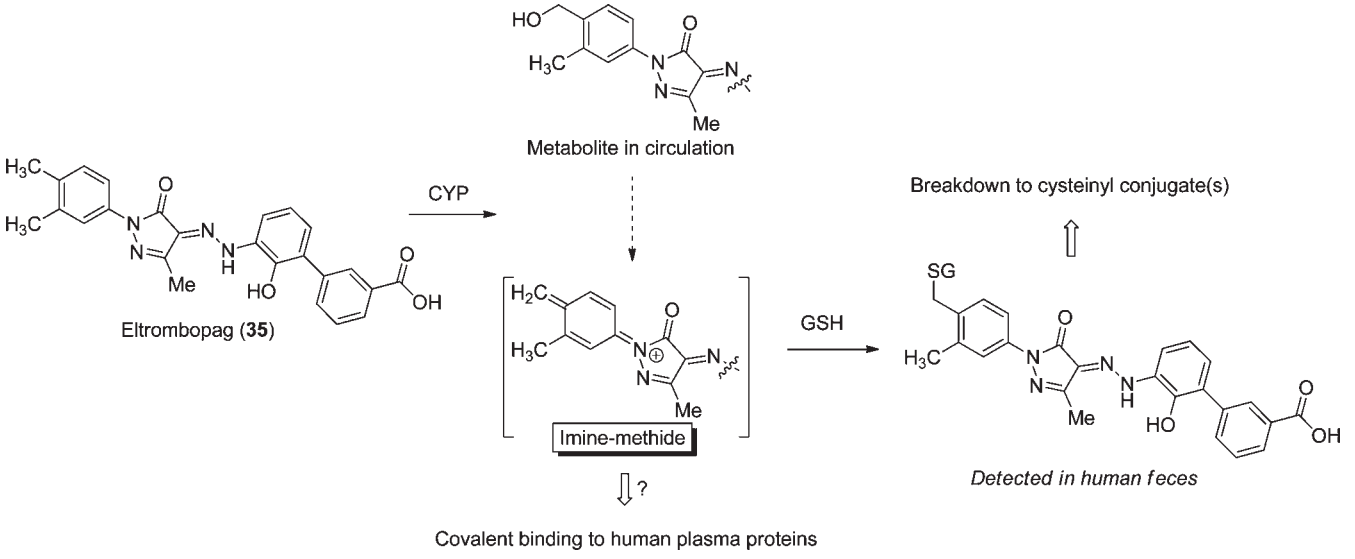
to an electrophilic epoxide intermediate. From the 37 drugs listed in Table 2, data for BSEP inhibition and/or mitochondrial dysfunction has been presented for nine compounds.

4.1. Acidic Compounds. Out of the five carboxylic acid drugs, four (chlorambucil (**33**), deferasirox (**34**), eltrombopag (**35**), and valproic acid (**36**)) contain structural alerts and information to qualify RM formation as a causative factor for toxicity exists for three (chlorambucil, eltrombopag, and valproic acid) of them. In humans, deferasirox (**34**) is primarily metabolized through glucuronidation of its phenol and carboxylic acid groups;[109] there is no evidence to suggest oxidation of the pendant phenol groups to RMs via the catechol−quinone pathway. The endothelin receptor antagonist ambrisentan (**32**) is a unique example of a low daily dose drug (10 mg) that previously carried a BBW for hepatotoxicity. Recent regulatory action has withdrawn the BBW on ambrisentan as a class effect due to evaluation of the clinical trial data and postmarketing safety information (http://www.fda.gov/Drugs/DrugSafety/ucm245852.htm). It is worth mentioning that ambrisentan does not feature a structural alert, appears to be devoid of RM formation, and is a very weak inhibitor of the BSEP transporter.[110] The mechanistic details for ambrisentan hepatotoxicity remain unclear at the present time.

Chlorambucil (**33**) is an aniline mustard derivative and is intrinsically electrophilic. Indiscriminate reactivity of this haloalkane with proteins and DNA can account for the bone marrow toxicity/blood dyscracias and mutagenic effects. Certainly, GSH alkylation by chlorambucil catalyzed by glutathione transferase has been established and is generally regarded as a detoxication pathway for most nitrogen mustards.[111]

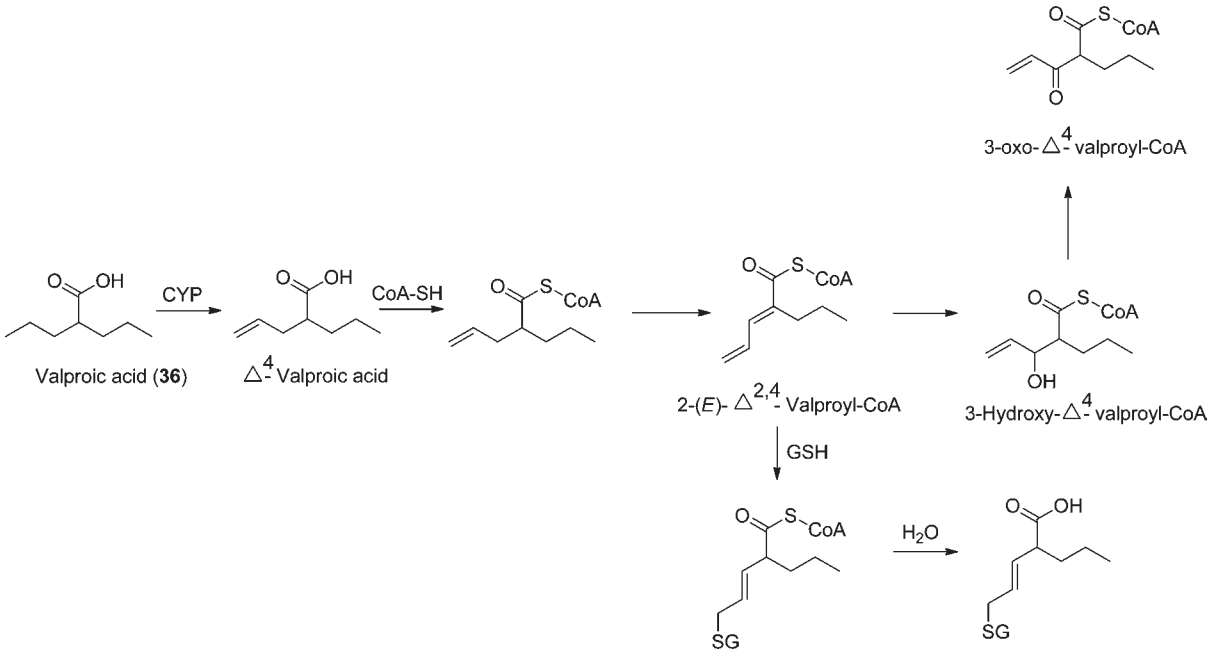
Evidence for RM formation with eltrombopag (**35**) has been obtained in human mass balance studies.[112] Absorbed eltrombopag was shown to be extensively metabolized, with feces containing ~20% of the administered dose as GSH-related conjugates. The GSH conjugates were formed via the addition of the thiol to a *para*-imine methide species that is formed via a CYP-mediated two−electron oxidation of the *para*-alkylaniline structural alert in the parent drug (Scheme 12). Low levels of covalently bound drug-related intermediates to plasma proteins were detected, which could potentially result from the reaction of the imine methide with proteins. The covalently bound material is thought

Scheme 12. ***In Vivo*** **Oxidative Bioactivation of the Thrombopoietin Receptor Agonist Eltrombopag in Humans**



dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Scheme 13. β-Oxidation of the Anticonvulsant Valproic Acid (36) to RMs



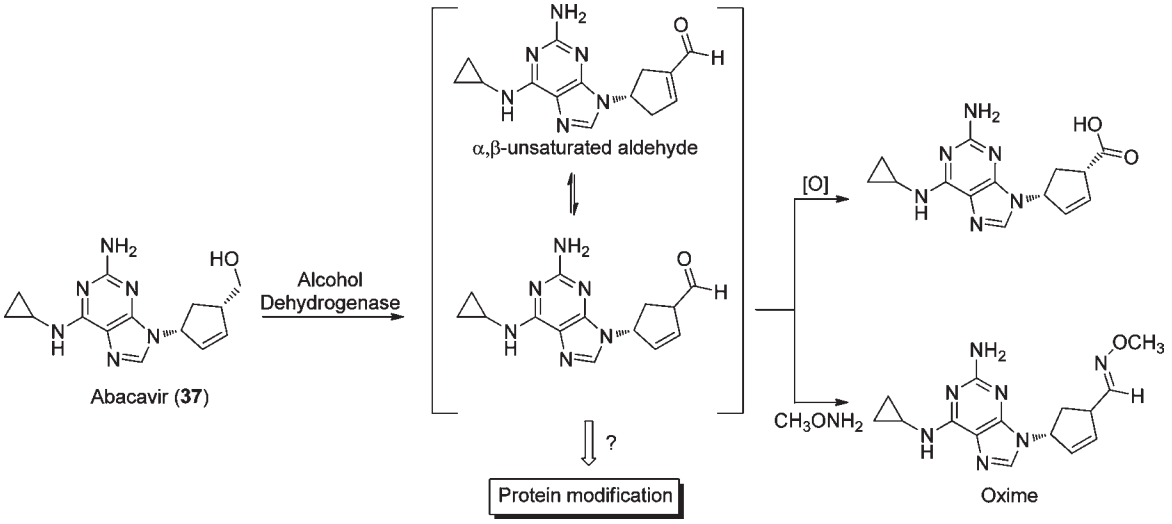
to contribute to the longer plasma elimination half-life of radioactivity.

Valproic acid (**36**) has been shown to be effective against a broad spectrum of seizure types since its introduction in 1967. However, the drug is associated with a rare, but serious effect involving irreversible liver failure (usually characterized by hepatic steatosis with or without necrosis), which has resulted in a BBW.[113] The involvement of toxic and potentially reactive valproic acid metabolites was first suggested by Gerber et al. on the basis of structural analogy with the known hepatotoxicant 4-pentenoic acid, which is associated with mitochondrial damage and impairment of fatty acid oxidation.[114] β-Oxidation in 4-pentenoic acid is believed to lead to 3-oxo-4-pentenoyl-CoA, a reactive, electrophilic species that is thought to alkylate 3-ketoacyl-CoA-thiolase, the terminal enzyme for β-oxidation, resulting in the mechanism-based inactivation of this enzyme complex. Studies with the terminal olefin metabolite of valproic acid (i.e., Δ^4-valproic acid) in perfused rat liver or in primates revealed products of β-oxidation as illustrated in Scheme 13.[115,116] Following conversion of Δ^4-valproic acid to its CoA derivative, sequential steps of β-oxidation lead to 2-(*E*)- $\Delta^{2,4}$-valproic acid, 3-hydroxy-Δ^4-valproic acid, and 3-oxo-Δ^4-valproic acid as the corresponding CoA derivatives. 2-(*E*)-$\Delta^{2,4}$-Valproic acid and 3-hydroxy-Δ^4-valproic acid (free acid forms) have been identified as metabolites in perfused rat liver and in primates *in vivo*. 3-Oxo-Δ^4-valproyl-CoA is believed to be the reactive, electrophilic species that binds covalently to the ketoacylthiolase protein resulting in its inactivation, while adducts derived from the reaction of GSH and *N*-acetylcysteine with 2-(*E*)- $\Delta^{2,4}$-valproyl-CoA in preclinical species and humans suggest a role for this RM in valproate toxicity.[117−120] While Δ^4-valproic acid was first detected as a minor metabolite in the plasma of epileptic children receiving valproic acid, much higher levels were detected in the serum of pediatric patients (the group most susceptible to valproic-acid-induced liver injury) than in either youths or adults, strengthening its potential role in toxicity.[118]

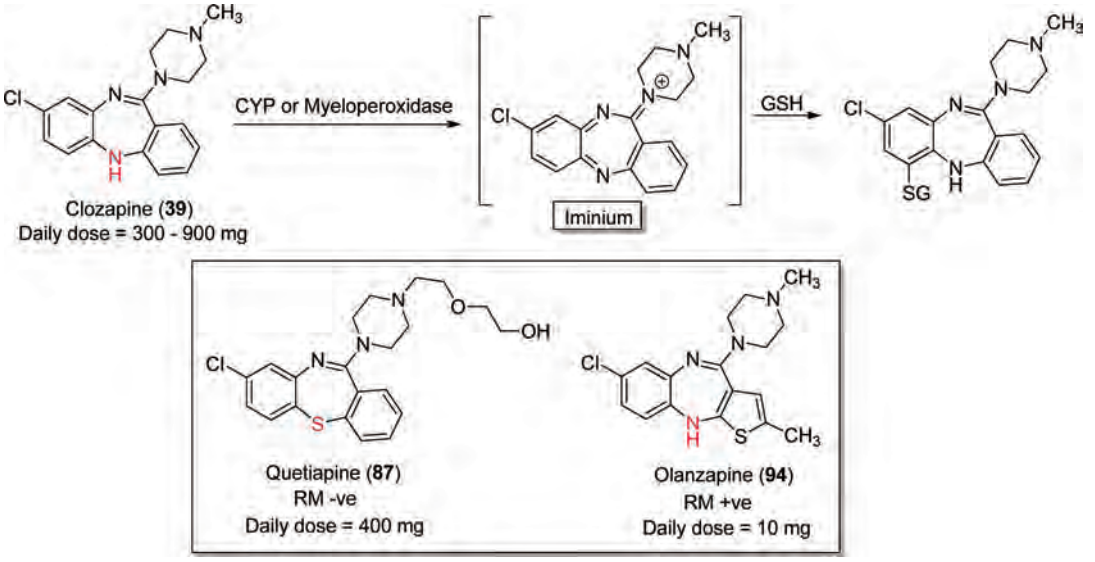
4.2. Basic Compounds. Eighteen out of the 22 basic drugs with a BBW label feature structural alerts, and evidence for RM formation has been presented for 13 compounds. Abacavir (**37**) is a nucleoside analogue reverse transcriptase inhibitor used to treat HIV and AIDS. While it is generally well tolerated, its use is associated with significant cases of hypersensitivity (e.g., fever, skin rash, etc.), which can be severe and, in rare cases, fatal. Hypersensitivity with abacavir has been linked to mutations in the HLA gene, particularly HLA-B*5701, for which genetic testing is now available in most western countries. Prior screening for the HLA-B*5701 mutation has been shown to reduce the incidence of abacavir hypersensitivity reactions.[1] Evidence for RM formation has been presented to explain the immune nature of abacavir IADRs. The major route of metabolism of abacavir in humans involves modifications to the primary β,γ-alcohol moiety resulting in a glucuronide conjugate and a carboxylic acid that is formed via the action of cytosolic alcohol dehydrogenases (Scheme 14).[121] *In vitro* incubations of [^{14}C]-abacavir in NAD-supplemented human cytosol results in protein covalent binding that is thought to be mediated by an electrophilic α,β-unsaturated aldehyde species.[121] Formation of the α,β-unsaturated aldehyde intermediate potentially could occur through an isomerization of the β,γ-unsaturated aldehyde species, formed in the course of the oxidative process. The characterization of an oxime derivative in cytosolic incubations of abacavir and methoxylamine (Scheme 14) provides support for the formation of the aldehyde intermediate.[121] The cyclopropylamine structural alert in abacavir does not play a role in the metabolism/bioactivation of this drug.

Amiodarone (**38**) is an antiarrhythmic drug with potentially life-threatening hepatotoxicity.[122] While the drug contains a diiodobenzene structural alert capable of forming RMs, several *in vitro* investigations have suggested that the stable *N*-dealkylated metabolites (mono-*N*-desethyl- and di-*N*-desethylamidarone)[123] may be responsible for amiodarone's hepatotoxicity through hepatocellular dysfunction.[124] Likewise, amiodarone and the *N*-deakylated metabolites are mitochondrial toxicants that

Scheme 14. Bioactivation of the Antiretroviral Agent Abacavir (37) in Human Liver Cytosol



Scheme 15. Structure−Toxicity Relationships in Benzodiazepine-Based Antipsychotic Agents, Clozapine (39), Quetiapine (87), and Olanzapine (94)

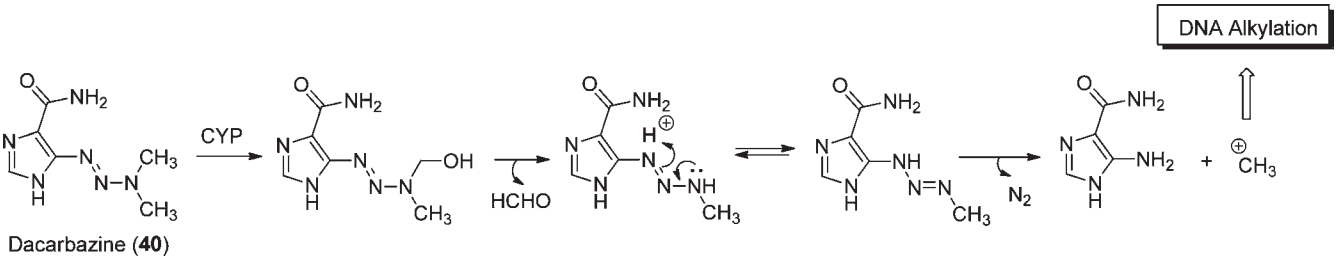


uncouple oxidative phosphorylation, inhibit β-oxidation, ultimately inducing apoptosis and necrosis.[125] Although amiodarone is a moderately potent inhibitor of BSEP activity *in vitro*,[93] rare but severe cases of intrahepatic cholestasis causing jaundice have been reported in patients typically on prolonged treatment with >400 mg/day.[126]

There is compelling evidence linking RM formation in the antipsychotic agent clozapine (**39**) with high incidences of blood dyscrasias and hepatotoxicity. Clozapine exhibits covalent binding to human neutrophil proteins *in vitro*, via MPO-mediated oxidation of the dibenzodiazepine ring to a reactive iminium ion, which covalently binds to the target tissues and also reacts with GSH (Scheme 15).[127−131] Proteins covalently modified with clozapine have been observed in neutrophils of patients being treated with clozapine, which reaffirms the relevance of the *in vitro* studies. With regards to structure−toxicity relationships, it is interesting to note that quetiapine (**87**), a structural analogue of clozapine, is not associated with IADRs despite administration at high doses (400− 800 mg). In fact, quetiapine (Table 4) was ranked 5th and 35th in terms of sales and dispensed prescriptions, respectively, in 2009. In quetiapine, the bridging nitrogen atom is replaced with a sulfur; consequently, this drug cannot form a reactive iminium (Scheme 15).[128] In contrast, olanzapine (**94**) (ranked 20th based on sales, Table 4) forms a reactive iminium metabolite very similar to the one observed with clozapine (Scheme 15), yet olanzapine is not associated with a significant incidence of blood dyscrasias. One difference between the two drugs is the daily dose; the maximum recommended daily doses of clozapine and olanzapine are 900 mg and 10 mg, respectively.

The clinical activity of the DNA methylating agent dacarbazine (**40**) is restricted to certain cancers such as Hodgkin's disease,

Scheme 16. Pathway of Dacarbazine (40) Metabolism That Leads to a DNA Alkylating Species



melanoma, and sarcoma. The common toxicities associated with this drug include hepatic necrosis and hemopoietic depression. The DNA alkylation capability of dacarbazine is dependent upon oxidative *N*-demethylation by several human CYP isoforms.[132] The proposed cascade of metabolic events leading to the DNA alkylating species is shown in Scheme 16. The initial product of dacarbazine oxidation, a carbinolamine derivative, produces the *N*-demethylated analogue that rapidly decomposes to the aminoimidazole derivative, the major plasma and urinary metabolite of dacarbazine in humans. The process can result in the liberation of the reactive methane diazohydroxide species, which could produce molecular nitrogen and a methyl carbocation, which is believed to be the alkylating species. The observation that dacarbazine toxicity is exacerbated in murine liver cells depleted of GSH[133] suggests a protective role for the thiol nucleophile possibly through scavenging of the alkylating species. It is also likely that indiscriminant alkylation of proteins triggers the hepatotoxicity associated with dacarbazine.

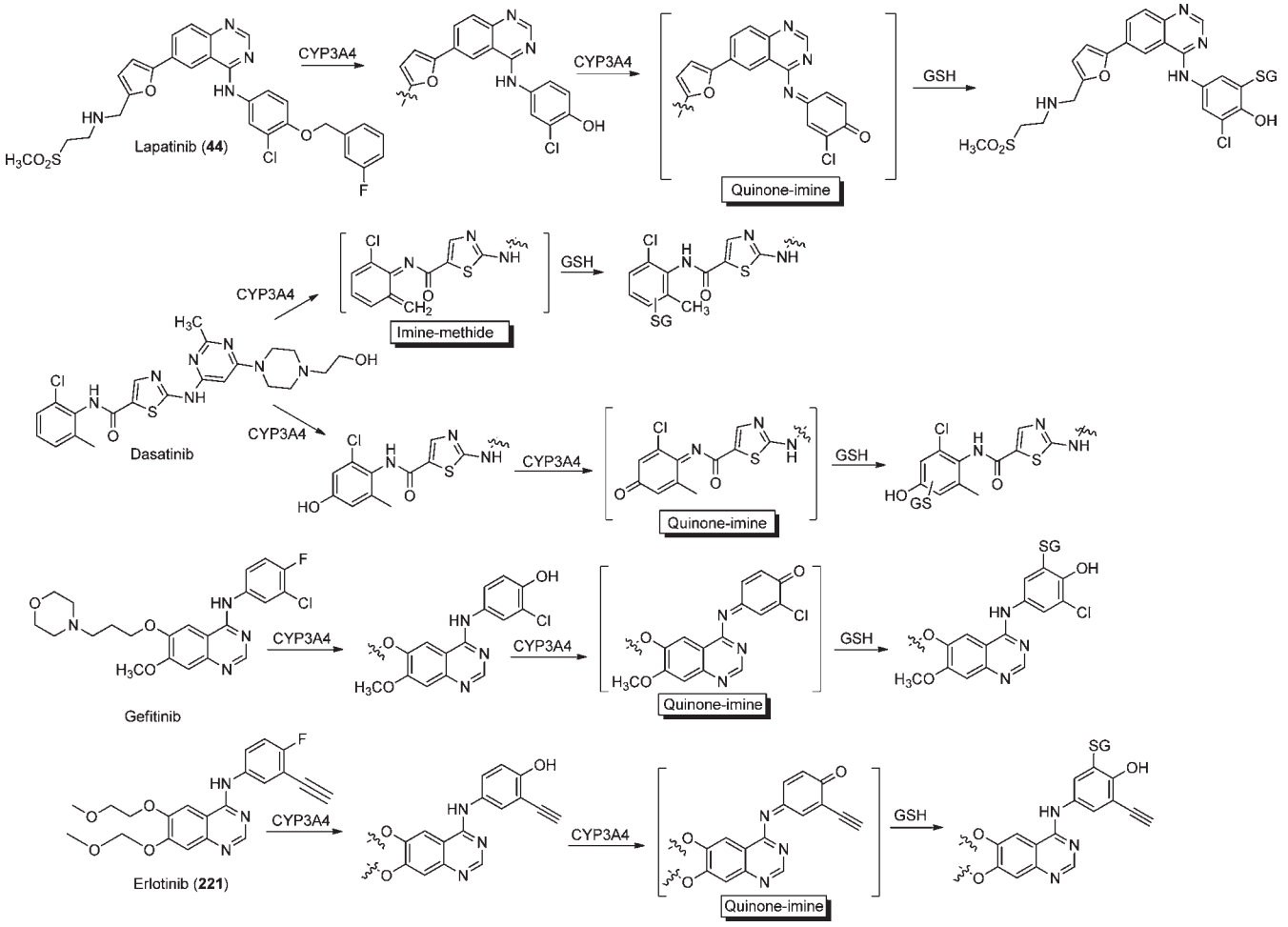
Insights into the biochemical basis for the hepatotoxicity associated with the antiarrhythmic agent flecainide (**41**) remain unclear. There are no formal reports on flecainide bioactivation to RMs, and its metabolism in human consists of innocuous biotransformation pathways involving *O*-dealkylation to the phenol metabolite followed by glucuronidation.[134] The cholestatic jaundice reported with flecainide use[135] implies a toxicity mechanism involving inhibition of BSEP, but this has not been examined. As in the case of iproniazid, bioactivation of the hydrazine group is thought to be the principal factor underlying the hepatotoxicity associated with the antituberculosis drug isoniazid (**42**).[136,137] A significant proportion of isoniazid metabolism in humans involves the formation of the *N*-acetylisoniazid metabolite by the polymorphic *N*-acetyltransferase (NAT) 2 enzyme.[138] The increased susceptibility of the slow-acetylator phenotype toward isoniazid-induced hepatitis suggests that *N*-acetylation is a detoxication pathway for isoniazid.[139] In slow acetylators, isoniazid metabolism could proceed predominantly through hydrolytic and oxidative pathways ultimately yielding the toxic chemical hydrazine.[139]

The antifungal agent ketoconazole (**43**) is associated with numerous cases of hepatitis in humans.[140] The biochemical pattern of ketoconazole-induced liver injury indicates extensive centrilobular necrosis and cholestasis. The absence of a manifestation of hypersensitivity in patients (e.g., eosinophilia, fever, and rash) indicates that hepatotoxicity is mediated through a direct insult to the liver by the parent compound and/or its metabolite(s). Ketoconazole is eliminated via hepatic metabolism in humans through pathways involving ring scission of the imidazole and piperazine rings, *O*-dealkylation, aromatic hydroxylation, and amide bond hydrolysis.[141] *N*-Deacetylketoconazole, derived from an amide bond cleavage in ketoconazole, is significantly more cytotoxic to hepatocytes than the parent compound. The observation that *N*-deacetylketoconazole cytotoxicity occurs in a time-dependent fashion and is suppressed by flavin monooxygenase (FMO) inhibitors suggests that downstream metabolites of *N*-deacetylketoconazole formed by FMO are ultimately responsible for the cytotoxic effects.[142,143] Upon *in vivo* administration to rats, [^3H]-ketoconazole exhibits covalent binding to liver proteins and depletes GSH in a time- and concentration-dependent fashion.[144] The identity of the RM of ketoconazole is unclear; it is believed that the rate-limiting step in ketoconazole bioactivation is a FMO-mediated piperazine ring *N*-oxidation of *N*-deacetylketoconazole to the corresponding hydroxylamine metabolite.[142,143] It is of interest to note that the piperazine ring has been widely used in drug design and is also found in the blockbuster drug sildenafil (**125**) (Table 1 in Supporting Information). In addition to the cytotoxic liabilities, ketoconazole also is a potent inhibitor of mitochondrial respiration and the BSEP transporter *in vitro*;[93] the latter phenomenon may explain the high degree of cholestasis leading to jaundice that is frequently reported with this drug.[140]

Lapatinib (**44**), the first oral dual tyrosine kinase inhibitor of ErbB-1 and ErbB-2, was approved by the FDA in 2007 for the treatment of advanced metastatic breast cancer. A BBW was released after postmarketing surveillance indicated an incidence of elevated liver enzymes and rare cases of liver-related fatalities that developed several days to a month after initiation of treatment. While the mechanism by which lapatinib causes hepatotoxicity is unknown, a circumstantial link to RM formation has been established.[145] The *O*-dealkylated metabolite of lapatinib in humans is a *para*-hydroxyaniline derivative that is oxidized to a reactive quinone-imine intermediate, which is trapped with GSH (Scheme 17). The bioactivation process is accompanied by mechanism-based inactivation of the CYP3A4 isoform, which is responsible for RM formation with lapatinib, and translates into supraproportional increases in systemic exposure of the drug upon repeated dosing in humans.[145] In addition to RM formation, lapatinib also exhibits potent BSEP inhibition[93] and collectively, the two liabilities, along with the high daily dose of 1250 mg, could be responsible for the hepatotoxic effects of this drug. There may also be a genetic risk associated with hepatotoxicity in women who carry the HLA-*DQA1**02:01 allele.[146]

It is interesting to note that the findings on lapatinib bioactivation parallels that of other structurally related tyrosine kinase inhibitors such as dasatinib, gefitinib, and erlotinib that have been shown to form reactive quinonoid metabolites (Scheme 17) and where hepatotoxicity is a possible side effect of treatment.[147−149] Gefitinib and erlotinib are *para*-fluoroanilines that are converted to the *para*-hydroxyaniline metabolites via a CYP-mediated ipso substitution on the fluorine substituent. Furthermore, gefitinib also exhibits BSEP inhibition with potency comparable to that of

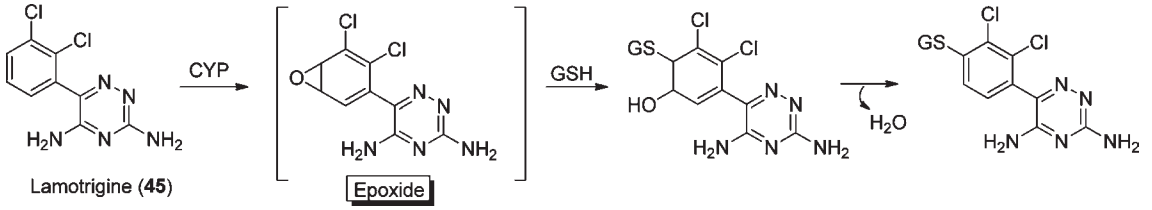
Scheme 17. Mechanism of RM Formation with the Tyrosine Kinase Inhibitors Lapatinib (44), Dasatinib, Gefitinib, and Erlotinib (221) in HLM



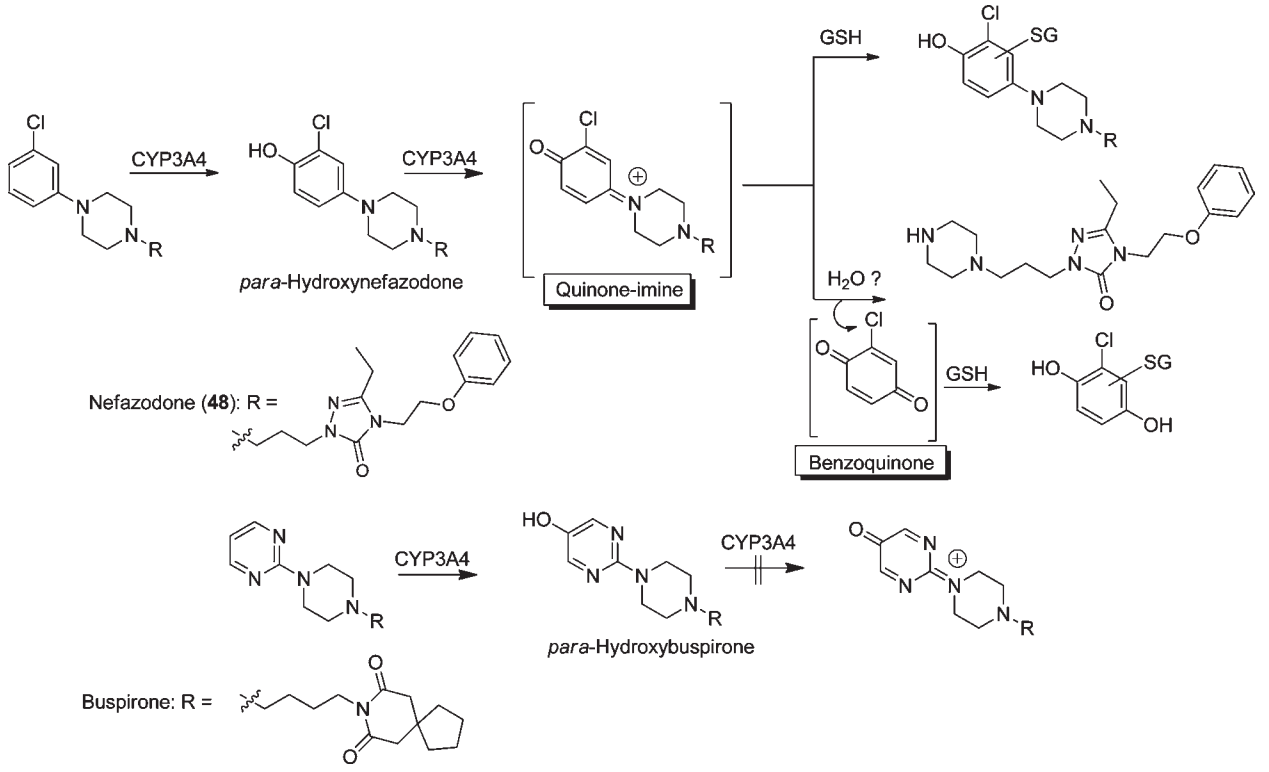
lapatinib.[93] It is interesting to note that despite the RM liability and potential for idiosyncratic hepatotoxicity, erlotinib (**221**) ranked 129th in 2009 in terms of its US sales (Table 2 in Supporting Information) implying that its therapeutic benefits in cancer treatment far outweigh the IADR risks. Pazopanib (**50**) is a potent and selective multitargeted receptor tyrosine kinase inhibitor that limits tumor growth and inhibits angiogenesis and has been approved for the treatment of patients with advanced renal cell carcinoma. In clinical trials, severe and fatal hepatotoxicity has been noted with pazopanib resulting in a BBW label. Whether the alkyl aniline structure in pazopanib succumbs to RM (imine-methide) formation remains unknown. While pazopanib is primarily cleared in humans via metabolism by CYP3A4, there are no reports on its biotransformation pathways. Pazopanib, however, has been reported to be a potent BSEP inhibitor in a manner similar to that of the other tyrosine kinase inhibitors.[93] BSEP inhibition may play a contributory role in the hepatotoxic effects of pazopanib given that the daily dose can be as high as 800 mg/day. Similar to pazopanib, sunitinib (**55**) is a multitargeted receptor tyrosine kinase inhibitor that has been approved for treatment of renal cell carcinoma as well as gastrointestinal stromal tumor. Compared to the other drugs listed in Table 2, sunitinib qualifies as a relatively low dose drug (50 mg QD); however, results from preapproval clinical trials indicate an equivocal hepatic risk profile for sunitinib resulting in a BBW label. Despite this liability, sunitinib also makes the top 200 drug list on the basis of total sales in 2009 (Table 2 in Supporting Information). While alerts (anilide and Michael acceptor) capable of forming RMs are present in sunitinib, its primary route of clearance in humans involves oxidative *N*-dealkylation to the *N*-desethyl metabolite, which has pharmacologic activity comparable to that of the parent drug (sunitinib package insert). There are no reports on BSEP inhibition and/or ability to cause cellular dysfunction resulting in toxicity.

Lamotrigine (**45**) has wide usage as an adjunct and monotherapy agent in the management of a wide range of epilepsy syndromes. Lamotrigine is generally well tolerated in most patients (ranked 109th and 156th in terms of total sales and dispensed prescriptions, respectively, in 2009), it is associated with a relatively high incidence (>10%) of immune-mediated hypersensitivity reactions including severe cutaneous ADRs. Rare cases of acute hepatotoxicity and blood dyscrasias have also been reported. While the diaminotriazine substituent in lamotrigine is the major site of biotransformation (humans eliminate lamotrigine principally as *N*-glucuronides in urine),[150] two separate studies have demonstrated the formation of an electrophilic epoxide intermediate on the dichlorophenyl ring that can be trapped by GSH (Scheme 18).[150,151] The finding that human epidermal keratinocytes

Scheme 18. Bioactivation of the Anticonvulsant Lamotrigine (45) in HLM and Human Epidermal Keratinocytes



Scheme 19. Bioactivation Pathways of the Hepatotoxin Nefazodone (48) in HLM: Comparison with Non-Hepatotoxic Buspirone



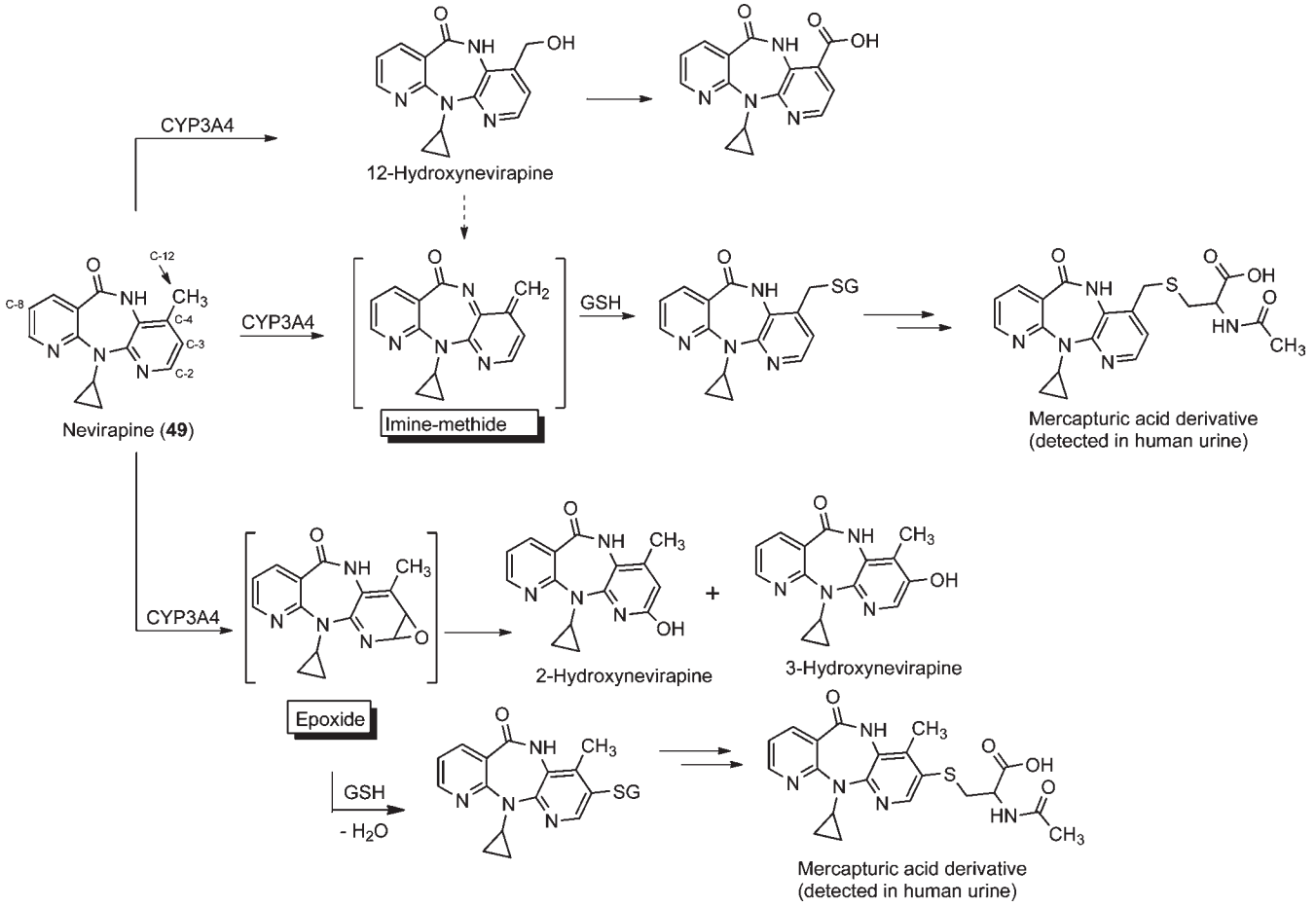
are capable of forming the same GSH conjugate identified in HLM strengthens the link between RM formation and the immune-mediated nature of the idiosyncratic cutaneous reactions.[152] Recently, it has been suggested that humans who carry the HLA-B*1502 allele are most susceptible to the cutaneous ADRs associated with lamotrigine and related anticonvulsant drugs such as carbamazepine (**60**).[153]

The biochemical basis for the idiosyncratic hepatotoxicity associated with the CCR5 antagonist maraviroc (**46**) is unclear. Maraviroc does not possess structural alerts, and there is no evidence suggestive of parent-mediated toxicity and/or RM formation with this drug.[154] Interestingly, clinical trials with a related CCR5 antagonist aplaviroc also revealed elevated liver enzymes (~70 times the upper limit of normal) in a small number of patients. Like maraviroc, aplaviroc is devoid of motifs capable of generating RMs, hence the underlying mechanisms for the hepatotoxicity associated with the two CCR5 antagonists remain unresolved.

Naltrexone (**47**), a broad opioid-receptor antagonist, is used for the treatment of alcohol dependence. Hepatotoxicity with naltrexone has not emerged as a clinical problem at the standard 50 mg dose, although at higher doses hepatoxicity presents significant concern. The margin of separation between the apparently safe dose of naltrexone and the dose causing hepatic injury appears to be only 5-fold or less. The principal route of naltrexone clearance in humans is reduction of the carbonyl moiety;[155] however, oxidative metabolism of the phenol functionality by CYP3A4 can also lead to the formation of a catechol/quinone intermediate as inferred from GSH trapping studies.[156] While the inhibitory potential against BSEP inhibition by naltrexone is unknown, the structurally related opioid antagonist naloxone displays very weak inhibition of BSEP activity (IC_{50} > 135 μM).[93]

Since its introduction in 1994, the antidepressant nefazodone (**48**) has been associated with numerous cases of idiosyncratic hepatotoxicity at therapeutic doses in the range of 200−400 mg/day, many of which required liver transplantation and/or resulted in fatalities. Nefazodone use has been banned in many countries in the European Union and Canada, and the generic version carries a BBW label in the United States. Evidence linking nefazodone hepatotoxicity with RM formation has been established; the *para*-hydroxyaniline architecture in *para*-hydroxynefazodone, a

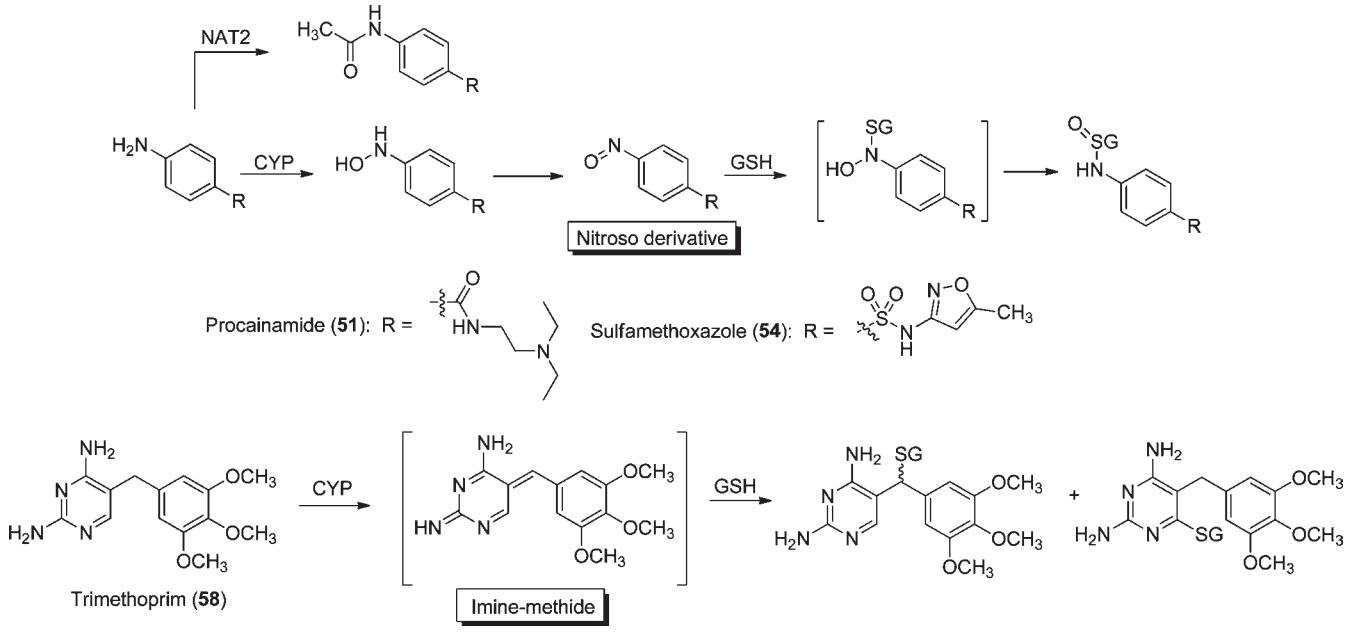
Scheme 20. Bioactivation of the Non-Nucleoside Reverse Transcriptase Inhibitor Nevirapine (49) in Humans



circulating metabolite of nefazodone in humans, is metabolized by CYP3A4 to electrophilic quinonoid species, which are trapped as GSH adducts (Scheme 19).[157,158] The nefazodone bioactivation pathway catalyzed by CYP3A4 is also accompanied by mechanism-based inactivation of the isozyme.[157] This *in vitro* observation is consistent with nefazodone's nonlinear pharmacokinetics (due to autoinactivation) and clinical drug−drug interactions (DDIs) with CYP3A4 substrates.[159] From a structure−toxicity perspective, it is interesting to note that the structurally related antianxiety drug buspirone is not associated with idiosyncratic toxicity despite decades of clinical use. While *para*-hydroxybuspirone represents a major circulating metabolite in humans (Scheme 19), failure to detect GSH conjugates in microsomal incubations of buspirone suggests that *para*-hydroxybuspirone does not succumb to RM formation in manner similar to that observed with *para*-hydroxynefazodone.[157] Absence of RM formation with buspirone in a manner similar to that of nefazodone is consistent with *ab initio* calculations, which suggest that a weaker resonance stabilization of the oxidation products and the greater acidity make *para*-hydroxybuspirone less favorable for the two-electron oxidation process. In addition to the RM liability, nefazodone (in its parent form) also exhibits mitochondrial toxicity and is a potent inhibitor of BSEP, features that are not shared by buspirone.[160,161] The reported cases of cholestasis with nefazodone can be potentially explained in part due to the inhibition of the BSEP transporter.[162]

Nevirapine (**49**) was the first non-nucleoside reverse transcriptase inhibitor approved for use in combination therapy of HIV infection and remains one of the most widely antiretroviral drugs in developing countries. Nevirapine is associated with two serious side effects, namely, cutaneous reactions and hepatotoxicity, and has resulted in a BBW.[163] In humans, the principal clearance pathway of nevirapine involves oxidative metabolism by CYP3A4 leading to several monohydroxylated isomers (2-hydroxy, 3-hydroxy, 8-hydroxy, and 12-hydoxynevirapine).[164] The 12-hydroxynevirapine metabolite is further oxidized to an electrophilic imine-methide species that is trapped with GSH (Scheme 20).[165−167] A competing metabolic fate of the 12-hydroxynevirapine metabolite is oxidation to the carboxylic acid derivative (Scheme 20). Formation of the 2- and 3-hydroxynevirapine metabolites requires the generation of an epoxide, which also can be intercepted by GSH.[165,166] *In vivo* proof for nevirapine bioactivation was established upon characterization of two isomeric nevirapine mercapturic acid conjugates in the urine of HIV-positive patients undergoing standard antiretroviral chemotherapy.[166] The mercapturates were unambiguously characterized as thioethers substituted at the C-3 and exocyclic C-12 positions of the methylpyrido ring of nevaripine, and are downstream metabolites of GSH conjugates. No RM formation is discerned with the cyclopropylamine structural alert in nevirapine. Finally, an examination of nevirapine's potential to inhibit BSEP indicates that the antiretroviral agent is a very weak inhibitor of the transporter.[93]

Scheme 21. RM Formation Pathways in Aniline-Containing Drugs Procainamide (51), Sulfamethoxazole (54), and the 2-Aminopyrimidine Derivative Trimethoprim (58)

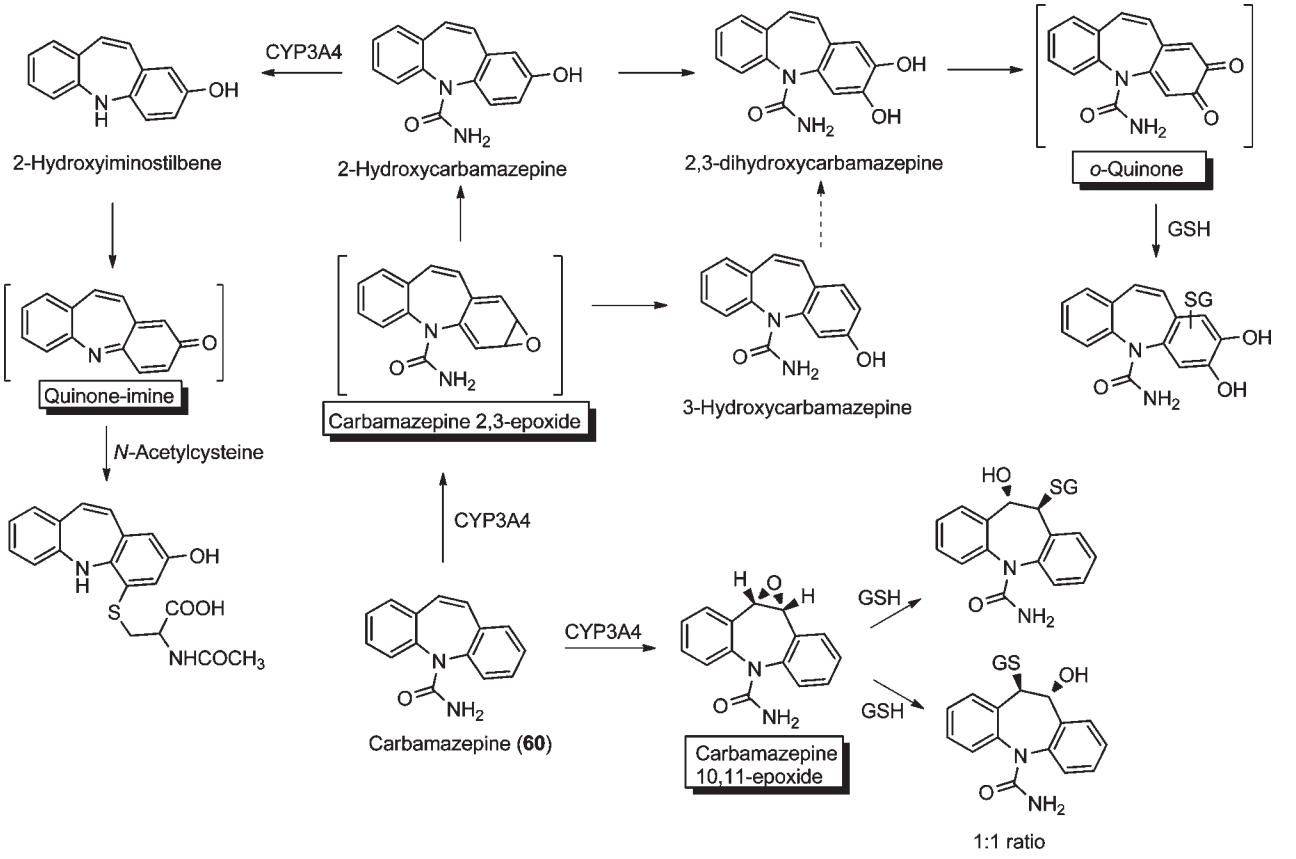


The relatively high incidence of immune-mediated toxicities (blood dyscrasias, cutaneous ADRs, and hepatotoxicity) associated with the antiarrhythmic agent procainamide (**51**) and the antibacterial drug sulfamethoxazole (**54**) have been linked with the oxidative bioactivation of the aniline motif to the corresponding hydroxylamine and nitroso intermediates (Scheme 21).[168−172] The nitroso metabolites covalently bind to microsomal protein, human neutrophils, and/or react with GSH to form the corresponding sulfinamide derivatives (Scheme 21). Sulfamethoxazole can also be oxidized in keratinocytes, implying that bioactivation and T-cell sensitization could occur directly on the skin leading to the severe cutaneous reactions observed with this drug.[171] In the case of procainamide and sulfamethoxazole, *N*-acetylation by NAT1 and the polymorphic NAT2 constitutes their major metabolic fate in humans.[173] Clinical studies with procainamide and sulfamethoxazole have indicated that patients of the rapid acetylator phenotype require a longer period of time to develop toxicity than slow acetylators, presumably due to the involvement of NAT2 in the elimination of the hydroxylamine metabolites of these drugs.[174,175] Procainamide and sulfamethoxazole are virtually devoid of BSEP inhibitory activity.[93] Trimethoprim (**58**) is a broad spectrum antibacterial agent that is frequently used in combination with sulfamethoxazole. While the hypersensitivity reactions observed with the combined drugs were thought to be related to sulfamethoxazole bioactivation, recent studies[176,177] have revealed the susceptibility of trimethoprim to form a reactive imine-methide species in HLM that is trapped by GSH (Scheme 21). RMs arising from the catechol/quinone pathway following *O*-demethylation have also been noted by Damsten et al. in HLM incubations of trimethoprim.[176] The bioactivation pathways are compatible with the primary metabolic pathways of trimethoprim in humans, which involve *O*-demethylation of the trimethoxybenzene group and hydroxylation of the methylene carbon.[178] Despite the safety liabilities associated with sulfomethoxazole/trimethoprim, it is interesting to note the two drugs sold in combined form as cotrimoxole were 44th in the list of the 200 most prescribed drugs (Table 1 in Supporting Information). The combination of pyrimethamine (**52**)/sulfadoxine (**53**) for the treatment of malaria is well tolerated when used as intermittent preventive treatment. However, pyrimethamine/sulfadoxine is no longer used as prophylactic treatment because of significant incidences of toxic epidermal necrolysis and Stevens−Johnson syndrome.[179] It is likely that the cutaneous ADRs are mediated through the bioactivation of the aniline functionality in sulfadoxine in a fashion similar to that in sulfamethoxazole. As such, there are no reports on biochemical and/or metabolic attributes of sulfadoxine and pyrimethamine that can shed light on the epidermal toxicity.

While the risk versus benefit of ticlopidine (**56**) has been shown to be positive for patients at increased risk for stroke, the use of the agent is limited by a significant incidence of blood dyscrasias and aplastic anemia. The mechanism for the IADRs is thought to involve an immunogenic mechanism involving a thiophene ring scission in ticlopidine to an electrophilic sulfenic acid metabolite in neutrophils.[180−182] The mechanism of ticlopidine bioactivation is analogous to that proposed for tienilic acid. Like ticlopidine, the antiarrhythmic agent tocainide (**57**) is associated with blood dyscrasias.[183] As such, the drug is eliminated via glucuronidation of the primary amine function yielding an *N*-carbamoyl glucuronide. The possibility that bioactivation of the 2,6-dimethylanilide motif could lead to RMs has not been published in the public domain.

4.3. Neutral Compounds. Seven out of the 10 neutral drugs with a BBW label feature structural alerts, and evidence for RM formation has been presented for five of these compounds. Information pertaining to RM formation has also been presented for the anticonvulsant drug felbamate (**64**), which does not contain a structural alert. The endothelin receptor antagonist bosentan (**59**) is a dose-dependent hepatotoxicant in humans. Analysis of clinical data from phase II hypertension trials with bosentan reveals an increase in the frequency of liver injury (2−11%) with increasing doses (100−2000 mg).[184] Furthermore,

Scheme 22. Bioactivation Pathways of the Anticonvulsant Drug Carbamazepine (60) in Humans

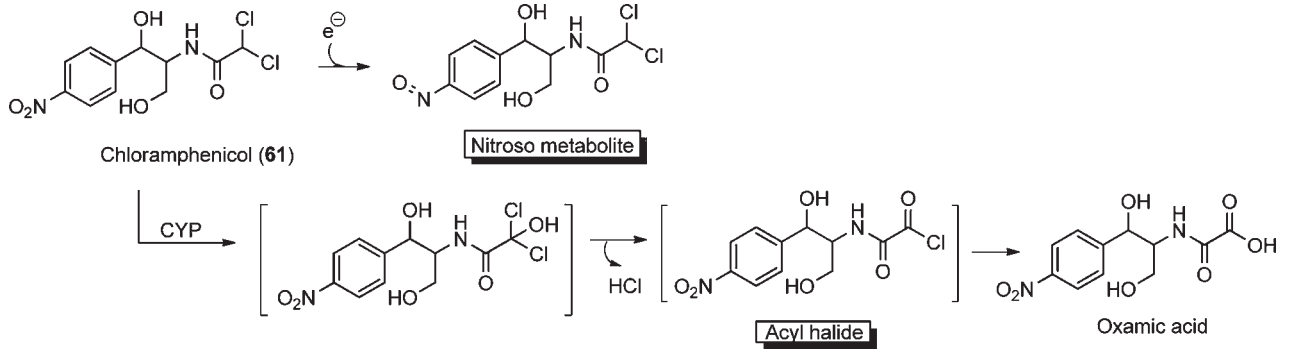


at daily doses of 2000 mg, all patients who exhibited increased liver enzymes also demonstrated increased serum bile salt levels indicative of cholestastic injury. While bosentan does not contain a structural alert and is not prone to RM formation, it should not be surprising that the high daily dose in combination with potent BSEP inhibition may confer additional risk for hepatotoxicity. Several studies have indicated that bosentan-induced liver injury is mediated, at least in part, by inhibition of BSEP through, intracellular accumulation of cytotoxic bile salts, and bile-salt-induced liver cell damage.[93,110,184,185]

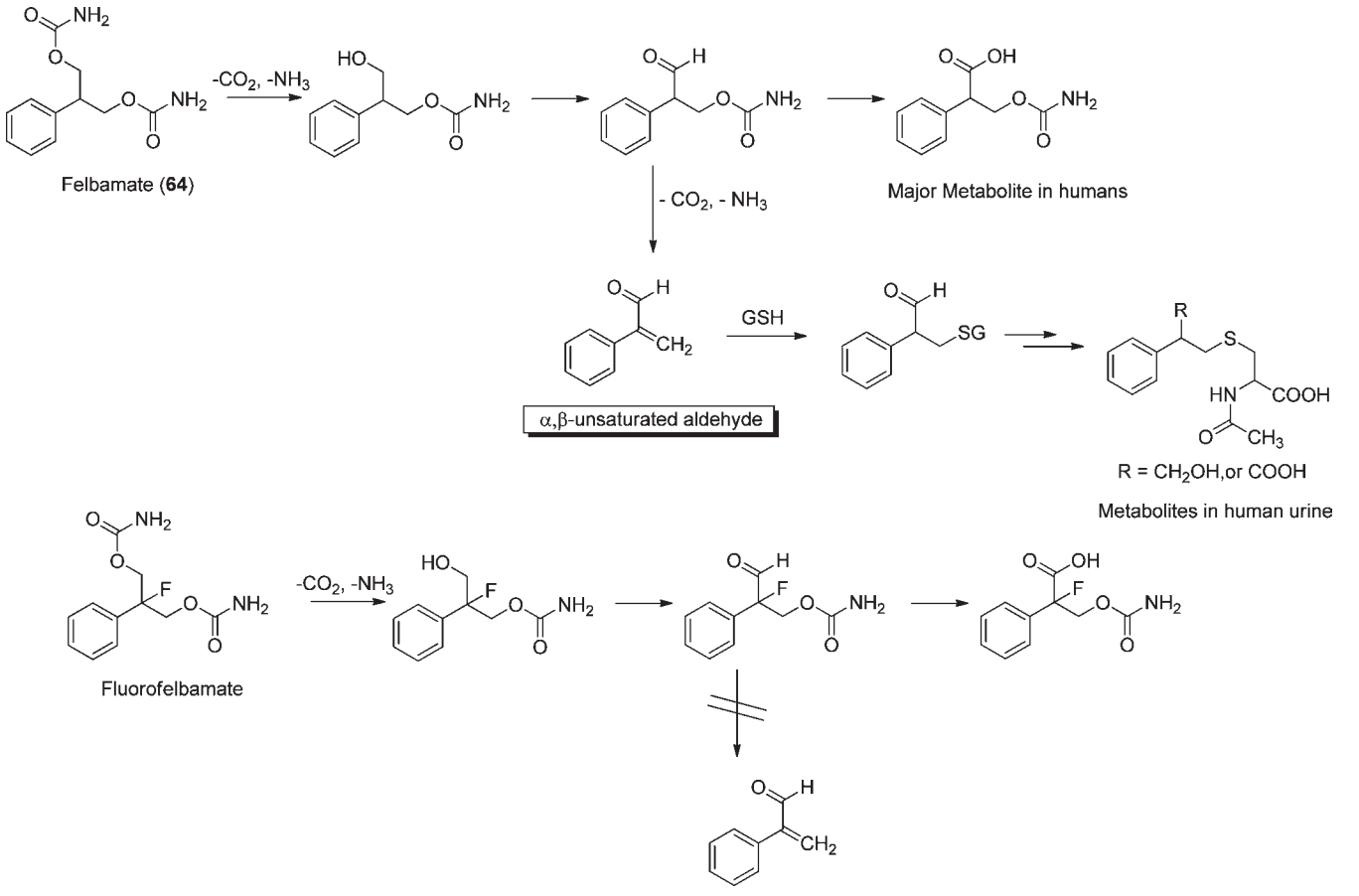
Introduced in the early 1960s, carbamazepine (**60**) has proven to be a highly effective agent for the treatment of epileptic seizures and psychiatric disorders. However, its therapeutic use has also been associated with a variety of serious IADRs, including skin rashes, aplastic anemia, hepatitis, and a severe, generalized drug-induced hypersensitivity reaction, which has been estimated to occur in 1 of 5000 patients.[186] Although the mechanism of carbamazepine-induced IADRs is not clear, the immune-mediated nature is thought to result from RM formation. Carbamazepine is extensively metabolized, and more than 30 metabolites have been identified in the urine of patients taking the drug.[187,188] The major biotransformation pathways include *N*-glucuronidation on the carbamoyl side chain of carbamazepine, formation of the active carbamazepine-10,11-epoxide metabolite, and hydroxylation on the aromatic rings leading to 2- and 3-hydroxycarbamazepine derivatives. Bioactivation of carbamazepine has been demonstrated *in vitro* with the formation of protein- and GSH-reactive metabolites in HLM.[189,190] Several RMs (Scheme 22) have been proposed as the reactive species responsible for the idiosyncratic toxicity associated with carbamazepine, including epoxides (carbamazepine 2,3-epoxide and carbamazepine 10,11-epoxide),[190−192] a quinone-imine metabolite derived from the metabolism of 2-hydroxycarbamazepine to 2-hydroxyiminostilbene intermediate,[193] and an *ortho*-quinone metabolite, which can form via oxidation of the catechol, 2,3-dihydroxy-carbamazepine.[194] The formation of each of these reactive species is compatible with the known urinary metabolites of carbamazepine in humans.[188] In addition, *in vitro* studies with recombinant human CYP3A4 have reported the covalent binding of carbamazepine with the isozyme leading to its suicide inactivation, a phenomenon that is consistent with the knowledge that sera from carbamazepine-hypersensitive patients often contain antibodies that recognize CYP3A proteins.[195,196]

The broad spectrum antibiotic chloramphenicol (**61**) has been used extensively in the practice of both human and veterinary medicine. The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which can occur in two distinct forms, bone marrow suppression that is dose-dependent and reversible and aplastic anemia, which is dose-independent, idiosyncratic and can lead to fatalities.[197] Biochemical evidence suggests a role for RMs in the pathogenesis of aplastic anemia with chloramphenicol. Although glucuronidation of the alcohol moieties is the major metabolic pathway of chloramphenicol in humans,[198] it has been suggested that a nitroso metabolite derived from the reduction of the nitrobenzene structural alert (Scheme 23) may be responsible for the adverse effects on bone marrow based on the *in vitro* observations involving covalent binding and toxicity of the metabolite to hematopoietic

Scheme 23. Bioactivation Pathways of the Antibiotic Chloramphenicol (61) in Humans and Animals



Scheme 24. Rational Chemical Approach to Circumvent Bioactivation Liability of Felbamate (64): Discovery of Fluorofelbamate

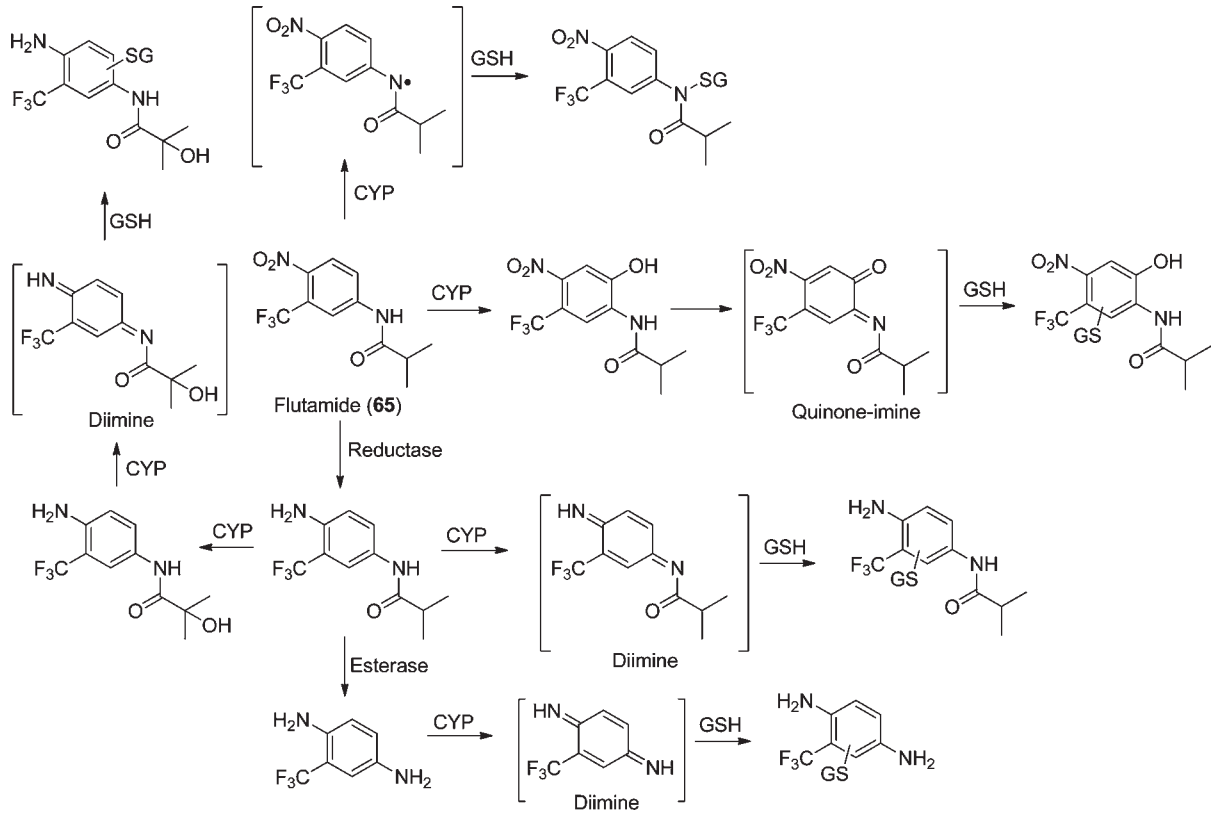


stem cells.[199,200] An alternative hypothesis centers on the oxidative metabolism of the dihaloalkane structural alert in chloramphenicol to a reactive acyl halide derivative (Scheme 23) that has been shown to covalently bind to liver microsomal proteins.[201,202] Additional support for this proposal arises from the detection of the corresponding oxamic acid metabolite, the product of the dehalogenation process, in human urine.[203]

Hepatic injury leading to fatalities have been noted with the long-term use of the muscle relaxant dantrolene (**62**) resulting in a BBW label. Dantrolene contains three structural alerts: nitrobenzene, furan, and hydrazine; one of the metabolic pathways of dantrolene in humans involves the reduction of the nitrobenzene ring to the corresponding aniline metabolite. The electrophilic nitroso intermediate in the nitroreductase pathway is known to form a sulfydryl conjugate in a manner similar to that of the aniline-containing drugs procainamide (**51**) and sulfamethoxazole (**54**).[204] Whether the nitroso species plays a role in toxicity remains unclear, especially since the idiosyncratic hepatic injury with dantrolene does not appear to involve a hypersensitivity component.[205] The possibility of BSEP inhibition remains to be evaluated as a contributing factor in the fatal cholestatic hepatitis associated with dantrolene.[206]

The anticancer agent docetaxel (**63**) is associated with several immune-mediated IADRs including hypersensitivity, skin toxicity,

Scheme 25. Known Bioactivation Pathways of the Antiandrogen Agent Flutamide (65) in Humans



blood dyscrasias, and hepatotoxicity (docetaxel package insert). Docetaxel is a cytotoxic chemotherapeutic agent and so is effectively a biologically damaging drug. Docetaxel is a cell-cycle-specific agent and is cytotoxic to all dividing cells in the body including tumor cells, hair follicles, and bone marrow. It is possible, therefore, that the IADRs are mediated, in part, by this cytotoxic activity. With reference to the liver injury phenomenon associated with the drug, it is interesting to note that docetaxel is a semisynthetic analogue of paclitaxel (**245**) (Table 2 in Supporting Information), which exhibits potent BSEP inhibition *in vitro*.[93] It is therefore possible that docetaxel possesses similar BSEP inhibitory characteristics to those of paclitaxel, but this remains to be determined.

Shortly after the approval of felbamate (**64**) in 1993, > 50 cases of aplastic anemia and hepatotoxicity were reported, as a result of which the FDA restricted the use of the anticonvulsant to patients already receiving the drug or to those refractory to other epilepsy treatments.[207] Indirect evidence linking RM formation *in vivo* with felbamate toxicity has been presented.[208−210] 3-Carbamoyl-2-phenylpropionic acid, an oxidized metabolite of felbamate, has been identified as the major metabolite of felbamate in humans (Scheme 24).[211] The intermediate 3-carbamoyl-2-phenylpropionaldehyde derivative is highly unstable (half-life <30 s) under physiological conditions and spontaneously degrades to the α,β-unsaturated aldehyde 2-phenylpropenal, which can react with GSH under nonenzymatic conditions (pH 7.4 buffer) and in the presence of glutathione transferases (Scheme 24). Evidence for the occurrence of this pathway *in vivo* has been demonstrated by the characterization of urinary mercapturic acid conjugates (derived from the reduced and oxidized forms of 2-phenylpropenal) following felbamate administration to human.[208,209] On the basis of the overall mechanistic information, fluorofelbamate was specifically designed to eliminate the RM liability of felbamate. The strategic placement of the fluorine atom on the benzylic position prevents the β-elimination process that affords the α,β-unsaturated aldehyde.[212] The major metabolic fate of fluorofelbamate in human hepatic tissue involves oxidation to a carboxylic acid metabolite (Scheme 24). Whether the absence of RM translates into a reduced risk of aplastic anemia and hepatotoxicity remains to be seen in long-term clinical trials with this drug.[212]

Flutamide (**65**), a widely prescribed nonsteroidal antiandrogen drug, has been shown to increase the survival time of prostate cancer patients in combination therapy with luteinizing hormone-releasing agonists or orchiectomy. However, a number of hepatotoxicity cases including rare incidences of severe liver dysfunction have been associated with the clinical use of this drug.[213,214] Several cases of blood eosinophilia have been observed in patients treated with flutamide, which indicates an immune-mediated toxicological outcome.[213,214] Flutamide undergoes extensive metabolism in humans primarily via hydroxylation on the isopropyl group to afford 2-hydroxyflutamide.[215,216] In addition to oxidative metabolism, the amide hydrolysis product 4-nitro-3-trifluoromethylaniline and its hydroxylamine derivative have been detected in the plasma and urine of patients.[216,217] Reduction of the nitro group to afford the corresponding aniline also has been observed as a minor metabolic pathway for flutamide in humans.[218] *In vitro*, flutamide covalently binds to hepatocytes and microsomal proteins in a CYP-mediated fashion, and the role of flutamide metabolism in the development of severe hepatotoxicity in rats also has been established.[219−221] Consistent with the CYP-dependent covalent binding, several GSH conjugates of flutamide and its downstream hydroxylated, reduced, and/or

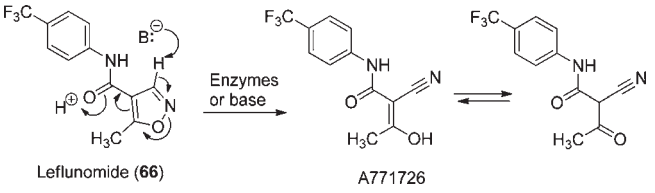
hydrolytic metabolites have been detected in human hepatic tissue (Scheme 25).[218,219,222,223] The identification of a mercapturic acid conjugate of hydroxylated flutamide in the urine of prostate cancer patients provides *in vivo* evidence for flutamide bioactivation and a causative role of RM in toxicity.[216]

Leflunomide (**66**) is a novel disease-modifying antirheumatic drug that is associated with idiosyncratic hepatotoxicity.[224] Serious hepatotoxicities reported include hepatitis, reactivation of viral hepatitis (especially hepatitis B), fulminant hepatic failure, jaundice, cholestasis, hepatomegaly, and hepatic cirrhosis. While it contains an anilide structural alert, its metabolic fate in humans involves N−O bond cleavage on the isoxazole ring to afford the active α-cyanoenol metabolite A771726 (Scheme 26).[225] While A771726 is electrophilic in nature, no GSH adducts derived from Michael addition to this metabolite have been detected. The inhibitory effects of leflunomide and its metabolite A771726 against BSEP remain to be characterized, particularly in relationship to the observed cholestatic injury.

Propylthiouracil (**67**) is an antithyroid drug used extensively in the treatment of Graves's disease. Its use is associated with several hypersensitivity reactions including blood dyscrasias, hepatitis, and lupus.[226] The mechanism of propylthiouracil-induced blood dyscrasias is postulated to involve RMs derived from oxidation of the thiourea structural alert (Scheme 27). Incubation of [^{14}C]-propylthiouracil in human polymorphonuclear leukocytes leads to covalent binding, mainly through disulfide bonds between protein and a RM of propylthiouracil.[227] Propylthiouracil is also oxidized by human neutrophils and recombinant MPO to propylthiouracil-disulfide, propylthiouracil-2-sulfinate, and the propylthiouracil-2-sulfonate derivative, which was shown to react with model sulfydryl nucleophiles such as *N*-acetylcysteine and 3-mercaptopropionic acid.[228] As shown in Scheme 27, propylthiouracil-sulfenyl chloride, which can be formed during MPO catalysis (H_2O_2 and Cl^-), also possesses the ability to react with cysteinyl residues on proteins leading to disulfide adducts. While glucuronidation is the major pathway of propylthiouracil metabolism in humans,[229] the possibility that the thiourea motif is converted to RMs in human hepatic tissue needs to be assessed especially in relation to the hepatotoxic effects of the drug in humans.

o-administration of the protease inhibitor tipranavir (**68**) with ritonavir (**219**) (Table 2 in Supporting Information) is associated with idiosyncratic hepatotoxicity that in some cases can be fatal.[230] However, in order to achieve effective tipranavir plasma concentrations, coadminstration with ritonavir is essential since ritonavir inhibits intestinal and hepatic CYP3A4 and intestinal *p*-glycoprotein that are involved in tipranavir disposition.[231] There are no formal reports that provide a causative role for tipranavir RMs in clinical toxicity; metabolism of tipranavir upon concomitant administration with ritonavir is minimal.[231] It is likely that the hepatotoxicity is mediated by ritonavir, which is associated with liver injury potentially through its high daily dose (600 mg twice daily), potent inhibitory effects on BSEP, and/or metabolism of its thiazole ring to RMs.[93,232]

Scheme 26. Metabolism of the Anti-Inflammatory Agent Leflunomide (66) to the Active α-Cyanoenol Metabolite A771726



5.0. STRUCTURAL ALERT AND RM ANALYSES FOR THE TOP 20 DRUGS IN THE US MARKET IN 2009, BY DISPENSED PRESCRIPTIONS

Table 3 lists the top 20 drugs in the US market categorized by virtue of dispensed prescriptions. Multiple ranks for a given drug

Scheme 27. Bioactivation of the Antithyroid Drug Propylthiouracil (67) by Myeloperoxidase Enzyme in Activated Neutrophils

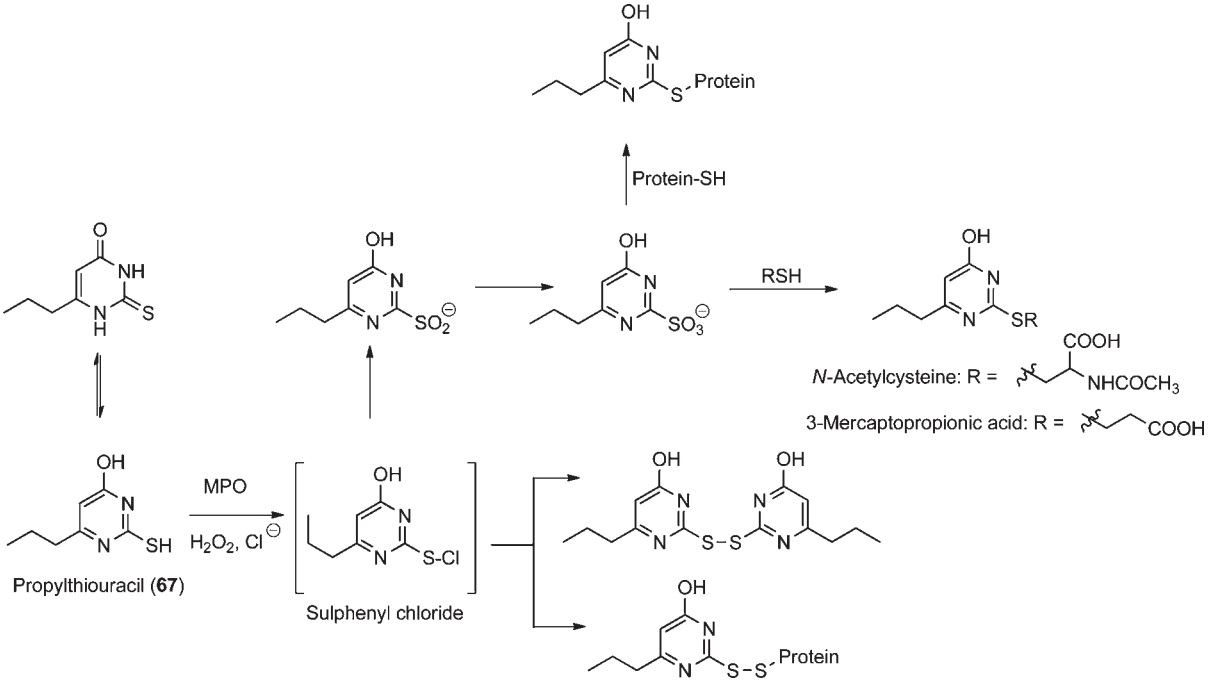
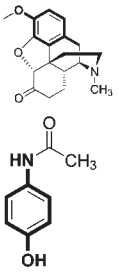
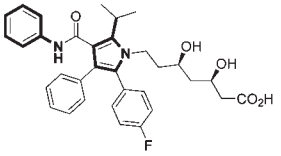
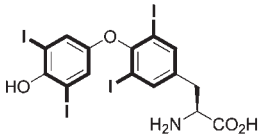
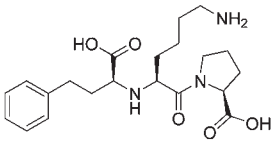
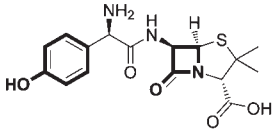
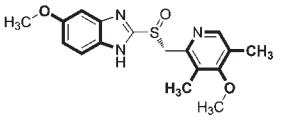
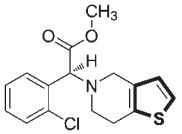
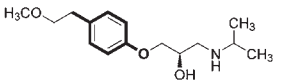
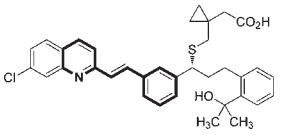
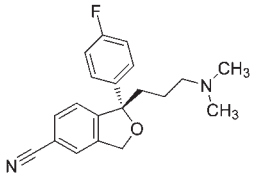
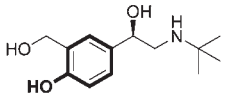
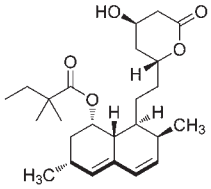
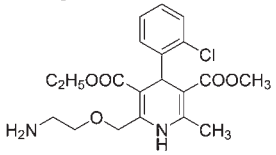
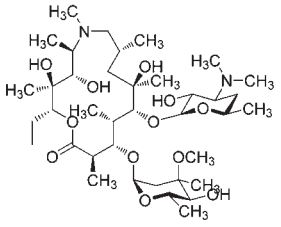
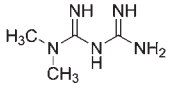


Table 3. Structural Alert and RM Analysis for the Top 20 Drugs in the US Market by Dispensed Prescriptions, 2009

Drug	Rank[a]	Indication	Phys. Chem.	MW	LogP	TPSA (Å^2)	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation
Hydrocodone (**69**) Acetaminophen (**70**) 	1,3,66	Analgesic / Anti-inflammatory	Base Neutral	299 151	1.12 0.49	38.77 49.33	40–4000	*p*-Alkylaromatic ether, *p*-Hydroxyanilide	Yes[7] (for acetaminophen)
Atorvastatin (Lipitor®) (**71**) 	2	Lipid Lowering	Acid	558	4.45	110	10–80	Anilide, 2-Alkylpyrrole	Yes[92]
Levothyroxine (Synthroid) (**72**) 	4,12, 18,103	Hyperthyroidism Pituitary TSH suppression	Neutral	776	3.50	92.78	0.1–0.125	Iodobenzene	N.D.[c]
Lisinopril (**73**) 	5,32, 74,81, 150	Antihypertensive	Neutral	405	-1.82	132.96	40	None	N.D.[c]
Amoxicillin (**74**) 	6	Antibacterial	Neutral	365	-2.19	132.96	2000	β-Lactam, Phenol	Yes[d,234,237]
Esomeprazole (Nexium®) (**75**) 	7	Gastresophageal reflux disease	Neutral	345	2.56	72.28	20–40	*p*-Alkoxyaniline, *o*-Alkylaromatic ether	Yes[d,240–242]
Clopidogrel (Plavix®) (**76**) 	8	Platelet aggregation inhibitor	Base	321	3.45	29.54	75	Thiophene	Yes[d,244–248]
Metoprolol (**77**) 	9,19, 88,170	Antihypertensive	Base	267	1.48	50.72	25–100	*p*-Alkylaromatic ether	N.D.[c]
Montelukast (Singulair®) (**78**) 	10	Antiasthma	Acid	585	8.47	69.89	10	Styrene	Yes[7]

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

Table 3. Continued

Drug	Rank[a]	Indication	Phys. Chem.	MW	LogP	TPSA (Å^2)	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation
Escitalopram (Lexapro®) (**79**) 	11	Antidepressant Generalized anxiety disorder	Base	324	3.13	36.26	10–20	None	N.D.[c]
Salbutamol / albuterol (Proair HFA®) (**80**) 	13,129,132	Antiasthma	Base	239	0.06	72.72	0.72	Phenol	N.D.[c]
Simvastatin (**81**) 	14,20, 26,69	Lipid lowering	Neutral	418	1.98	72.83	5–80	Olefin	Yes[254,255]
Amlodipine (**82**) 	15,38	Antihypertensive	Base	408	3.43	99.88	5–10	None	N.D.[c]
Azithromycin (**83**) 	16,29, 98	Antibacterial	Base	749	2.64	180.08	500–2000	None	N.D.[c]
Metformin (**84**) 	17,87, 178	Antidiabetic	Base	129	- 1.63	88.99	2550	None	N.D.[c]

[a] Multiple ranks imply that generic forms of the drug are marketed by multiple vendors. [b] Top end of the daily dose is the maximum recommended dose. [c] N.D., not determined (no published accounts in the primary literature). [d] Requires RM formation for primary pharmacology.

in the table imply that generic forms of the drug are marketed by more than one vendor. Of the top 15 drugs by dispensed prescriptions (hydrocodone (**69**)/acetaminophen (**70**) combination, metoprolol (**77**), and simvastatin (**81**) appear twice in the list, whereas levothyroxine (**72**) appears thrice in the list of 20 drugs), 10 (55%) were flagged for the presence of structural alerts, and evidence for bioactivation leading to RMs has been provided in 5 out of the 10 cases. In fact, two of the most prescribed drugs, esomeprazole (nexium) (**75**) and clopidogrel (plavix) (**76**), require RM formation for their pharmacological activity; while amoxicillin (**74**) is an affinity label that does not require bioactivation for the irreversible acylation of its target

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

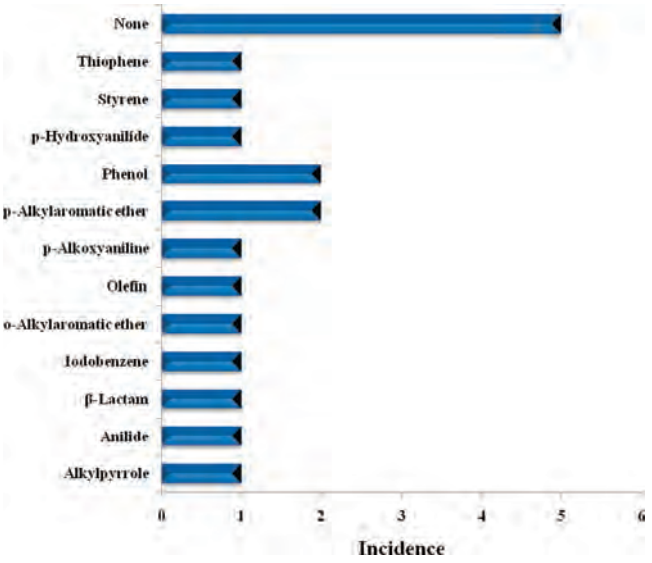


Figure 5. Structural alert analysis in the top 20 drugs in the US market by dispensed prescriptions in 2009.

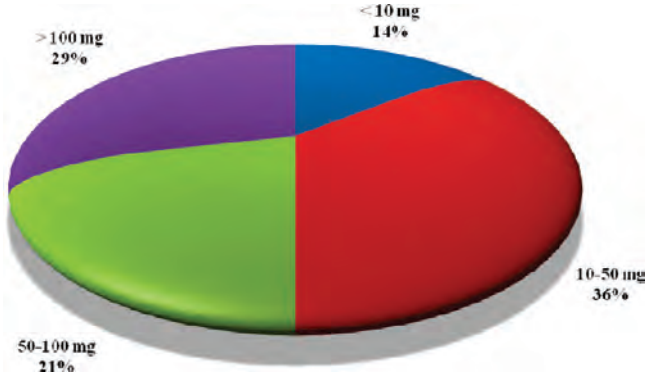
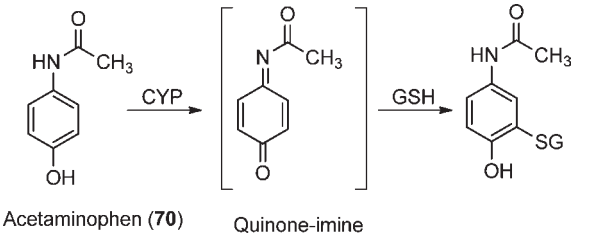


Figure 6. Maximum recommended daily dose assessments for the top 20 drugs in the US market by dispensed prescriptions in 2009.

enzyme. Substituents (phenol, alkylaromatic ether, hydroxyanilide, anilide, etc.) associated with the formation of electrophilic quinones, quinone-imines, and quinone-methides were identified as alerts in several instances (Figure 5). The drugs are categorized into three groups, including two acids, eight bases, and six neutral compounds. Similar to the situation with recalled drugs and drugs associated with BBW, the top 20 prescribed drugs depict a broad range of MWs (129−808 Da), log *P* values (−2.19 to +8.47), and TPSAs (36−180 Å^2). With the exception of acetaminophen (**70**) (combined with hydrocodone), amoxicillin (**74**), azithromycin (**83**), and metformin (**84**), the maximum recommended daily doses of the remainder of the drugs in Table 3 are 100 mg or less (in most cases, the pharmacologically effective doses are significantly lower) (Figure 6). For instance, the maximum recommended daily dose of atorvastatin (**71**) is 80 mg, but most patients demonstrate significant cholesterol lowering at the 10−20 mg daily doses. This trend presents a significant contrast with the high daily doses of drugs associated with toxicity. Of the top 20 prescribed medicines, lisinopril (**73**), escitalopram (**79**), amlopidine (**82**), azithromycin (**83**), and metformin (**84**) do not contain structural alerts.

Scheme 28. Bioactivation of the Anti-Inflammatory Agent Acetaminophen (70) by CYP Enzymes



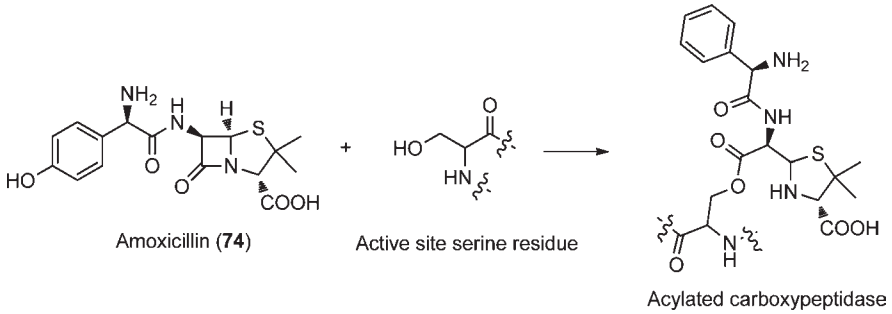
Acetaminophen (**70**) Quinone-imine

The most widely dispensed retail prescription in the US (128 million dispensed prescriptions) in 2009 was the pain reliever Vicodin that combines the narcotic hydrocodone (**69**) with acetaminophen (**70**). In that same year, a US FDA advisory panel voted by a narrow margin to advise the FDA to remove Vicodin and the related painkiller, Percocet (oxycodone/acetaminophen), from the market because of "a high likelihood of overdose from prescription narcotics and acetaminophen products". The panel cited concerns over the potential for liver damage from the acetaminophen component present in these combinations. The acetaminophen-narcotic combination has special hazards if patients develop a tolerance to the narcotic and increase their daily intake, leading to a potentially fatal acetaminophen overdose. Each year, acetaminophen overdose is linked to about 400 deaths and 42,000 hospitalizations. At the recommended dosing regimen, the reactive quinone-imine metabolite of acetaminophen is detoxified by GSH stores in the liver (Scheme 28). However, an acute overdose of acetaminophen depletes the GSH pool, and as a result, the quinone-imine species accumulates in the liver, causing hepatocellular necrosis and possibly damage to other organs.[21] Severity of hepatotoxicity after a single acute overdose is predicted by plasma acetaminophen levels. The antidote to acetaminophen overdose is the thiol nucleophile *N*-acetylcysteine, which replenishes hepatic GSH and prevents further damage to the liver.

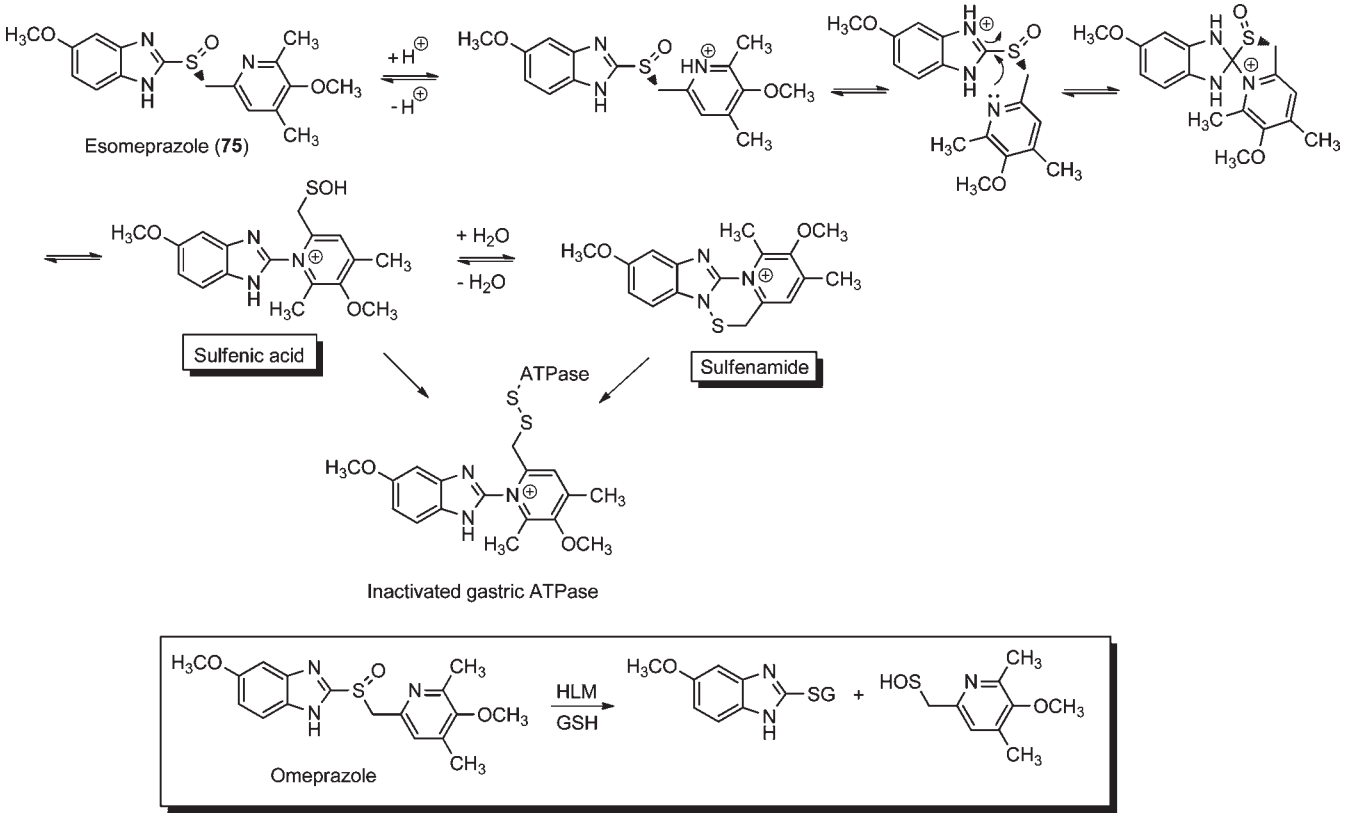
In the case of the lipid-lowering agent atorvastatin (**71**), CYP3A4 catalyzed monohydroxylation on the acetanilide structural alert results in the formation of the corresponding *ortho*- and *para*-hydroxyacetanilide metabolites, which can be potentially oxidized to reactive quinone-imine species.[233] Consistent with this hypothesis, atorvastatin has been shown to covalently bind to HLM in a NADPH-dependent fashion.[92] The exact nature of the RM that is responsible for protein covalent binding is unkown at the present time. The excellent safety record of atorvastatin in relation to mechanism-independent IADRs can be potentially explained by the low efficacious daily dose of 10−20 mg. In general, liver toxicity has been a concern since the initial introduction of statins, but several clinical trials have shown that statins are safe to use for the prevention of coronary disease and death, even in the setting of chronic liver disease. Irreversible liver damage leading to death or liver transplantation appears to be extremely uncommon with statins. In fact, the incidence of liver enzyme elevations in the statin-treated population has not been consistently different than in placebo-treated patients.

Amoxicillin (**74**) belongs to the class of β-lactam antibacterial agents whose bacteriocidal action is directly attributable to their ability to react with the serine-type D-Ala-D-Ala carboxypeptidase. This enzyme is a serine protease involved in the bacterial synthesis of the peptidoglycan layer in the cell wall. All β-lactam antibacterial

Scheme 29. Irreversible Acylation of Serine-Type D-Ala-D-Ala Carboxypeptidase by Amoxicillin (74)



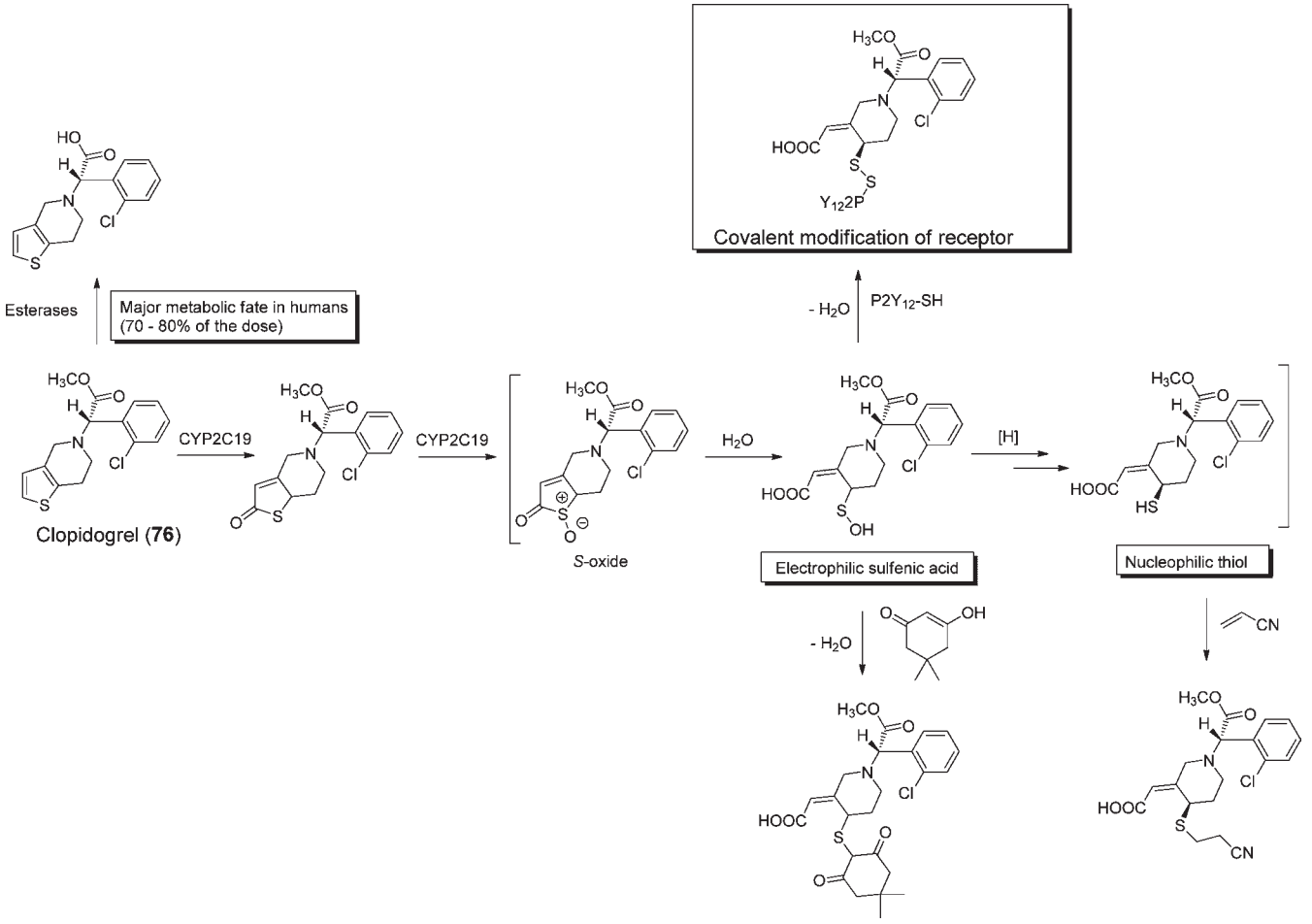
Scheme 30. Irreversible Inactivation of Gastric ATPase by Esomeprazole (75)



agents including amoxicillin, irreversibly acylate the active site serine, forming a serine ester linked adduct (Scheme 29).[234] Although β-lactam drugs are generally well tolerated, they are also frequently associated with IADRs (e.g., drug allergy and anaphylaxis). For instance, amoxicillin is associated with a very low rate of mild hepatocellular and cholestatic injury.[235] The acute nature of the treatment most likely aids the tolerability, considering that the daily doses of β-lactam antibiotics are very high. The IADRs associated with β-lactam antibacterial agents such as penicillin have been studied in much detail. In some patients, indiscriminant acylation of free amino and sulfydryl groups on proteins via a nonenzymatic β-lactam ring scission leads to an immune response against the penicillin−protein adduct, and if the antibody response generates sufficient IgE antibodies, a severe allergic reaction such as anaphylaxis can occur.[236−238] On the basis of this knowledge, it was possible to develop a test for penicillin allergy in which penicillin bound to a lysine polymer, when injected on the skin, can cause degranulation of mast cells leading to a local wheal and flare response. The mechanism of penicillin-induced allergic reactions fits the hapten hypothesis where the chemical reactivity of the penicillin allows it to generate a hapten. In fact, one of the principal metabolites of amoxicillin in humans is derived from the hydrolysis of the β-lactam ring.[239]

Proton transport by the gastric H^+/K^+ ATPase is the final step in gastric acid secretion. Proton pump inhibitors such as esomeprazole (nexium, **75**), which are used to treat gastresophageal reflux disease, elicit their pharmacological effects through covalent modification of the gastric ATPase via reaction with one or more cysteine residues.[240] The mechanism of covalent modification by proton pump inhibitors has been extensively studied.[241]

Scheme 31. CYP-Mediated Bioactivation of Clopidogrel (76) into a Reactive Sulfenic Acid Species That Is Responsible for the Primary Pharmacology



As illustrated with esomeprazole, proton pump inhibitors are prodrugs that transform under the acidic environment of the stomach to a spiro intermediate, which then undergoes rearomatization with elimination to a sulfenic acid derivative followed by subsequent dehydration to a tetracyclic sulfeneamide analogue. The sulfenamide intermediate reacts irreversibly with an active site cysteine in gastric ATPase to form an adduct and leads to inactivation of the proton pump (Scheme 30). RM formation is acid catalyzed and occurs locally at the site of action; therefore, systemic exposure to RMs (sulfenic acid and sulfenamide) is unlikely to be achieved. However, it is interesting to note that omeprazole (**112**, the racemic form of esomeprazole, Table 1 in Supporting Information) and related proton pump inhibitors are also capable of reacting with GSH in HLM in a non-NADPH-dependent fashion suggesting that these compounds may be intrinsically electrophilic.[242] The detection of the corresponding mercapturic acid analogue (derived from the downstream degradation of the GSH adduct shown in Scheme 30) in rat urine following omeprazole administration indicates the existence of this pathway *in vivo*.[243] From a toxicological standpoint, the relevance of the GSH displacement reaction remains unclear, especially given the low daily efficacious doses and the excellent safety record of esomeprazole and related proton pump inhibitors in humans. Finally, there is no evidence to suggest CYP-mediated RM formation on the *para*-alkoxyaniline and *para*-alkylaromatic ether structural alerts in esomeprazole in HLM.[244]

The blockbuster cardiovascular drug and $P2Y_{12}$ purinoreceptor antagonist clopidogrel (Plavix) (**76**) was first launched in 1999 in the U.S. for the reduction of atherosclerotic events in patients with stroke, myocardial infarction, or peripheral arterial diseases. A prerequisite for the beneficial pharmacologic activity of clopidogrel is its conversion by CYP enzymes to a RM that inhibits platelet aggregation by irreversibly inhibiting the $P2Y_{12}$ receptor in platelets.[245] On the basis of *in vitro* metabolism studies, it has been speculated that the active metabolite of clopidogrel is a thiol derivative, which forms a covalent disulfide linkage with a cysteinyl residue on the $P2Y_{12}$ receptor in platelets (Scheme 31).[246,247] However, as shown recently by Dansette et al.,[248] the molecular species that actually modifies the receptor almost certainly is the sulfenic acid intermediate (an electrophile) and not the thiol, which is nucleophilic in nature. The electrophilic sulfenic acid species derived from CYP2C19-mediated thiophene ring scission has been trapped in HLM using dimedone as a nucleophile, whereas the nucleophilicity of the thiol metabolite is evident from the characterization of a thioether metabolite following reaction with the exogenously added electrophile acrylonitrile (Scheme 31).[248] Overall, these observations with clopidogrel pose a fundamental question from a structure−toxicity perspective: Why is clopidogrel not

Table 4. Structural Alert and RM Analysis for the Top 20 Drugs in the US Market by Sales, 2009

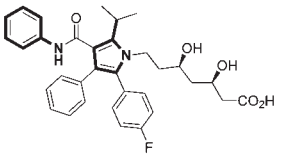
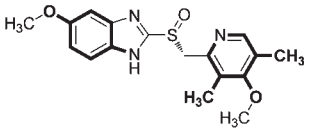
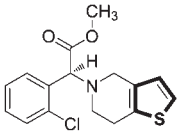
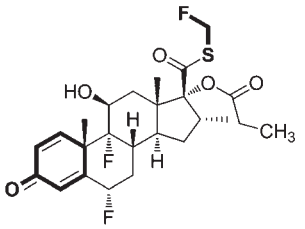
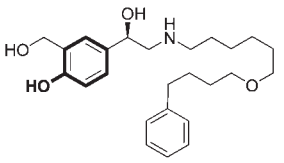
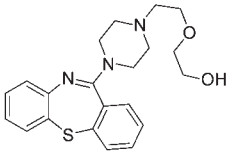
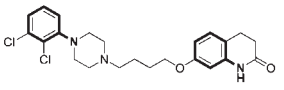
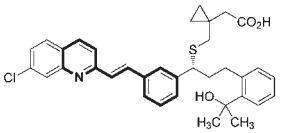
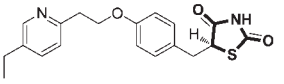
Drug	Rank	Indication	Phys. Chem.	M W	Log P	TPSA (Å^2)	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation
Atorvastatin (Lipitor®) (**71**) 	1	Lipid Lowering	Acid	558	4.45	110	10–80	Anilide, 2-Alkylpyrrole	Yes[92]
Esomeprazole (Nexium®) (**75**) 	2	Gastresophageal reflux disease	Neutral	345	2.56	72.28	20–40	*p*-Alkoxyaniline, *o*-Alkylaromatic ether	Yes[d,240–242]
Clopidogrel (Plavix®) (**76**) 	3	Platelet aggregation inhibitor	Base	321	3.45	29.54	75	Thiophene	Yes[d,244–248]
Fluticasone (**85**) /Salmeterol (**86**) (Advair® Diskus)  	4	Antiasthma	Neutral Base	500 415	2.29 3.06	80.67 81.95	1.0 0.1	Michael Acceptor, Thioester, Alkylhalide Phenol	Yes[257,258]
Quetiapine (Serequel®) (**87**) 	5	Schizophrenia	Base	383	2.99	48.30	400	None	No[128]
Aripiprazole (Abilify®) (**88**) 	6	Schizophrenia Antidepressant	Base	448	5.31	44.81	10–15	Aniline, Anilide	Yes[259]
Montelukast (Singulair®) (**78**) 	7	Antiasthma	Acid	585	8.47	69.89	10	Styrene	Yes[7]
Pioglitazone (Actos®) (**89**) 	8	Antidiabetic	Neutral	356	2.97	67.76	15–45	Thiazolidinedione	Yes[103]

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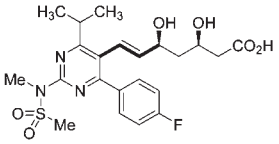
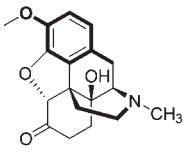
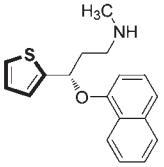
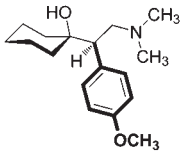
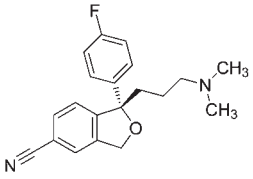
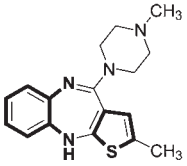
Table 4. Continued

Drug	Rank	Indication	Phys. Chem.	M W	Log P	TPSA (Å^2)	Daily Dose[b] (mg)	Alerts	Reactive Metabolite Formation
Etanercept (Enbrel®)[a]	9	Anti-inflammatory							
Epoetin Alfa (Epogen®)[a]	10	Anti-anemia							
Inflixmab (Remicade®)[a]	11	Anti-inflammatory							
Rosuvastatin (Crestor®) (**90**) 	12	Lipid lowering	Acid	481	1.89	139.86	5–40	Olefin	N.D.[c]
Bevacizumab (Avastin®)[a]	13	Anticancer							
Pegfilgrastim (Neulasta®)[a]	14	Anticancer Antiinfective							
Oxycodone (Oxycontin®) (**91**) 	15	Analgesic	Base	315	-0.03	59.00	40–160	*p*-Alkylaromatic ether	N.D.[c]
Duloxetine (Cymbalta®) (**92**) 	16	Antidepressant Generalized anxiety disorder	Base	297	4.25	21.26	60	Thiophene	Yes[262]
Venlafaxine (Effexor XR®) (**93**) 	17	Antidepressant	Base	277	3.20	32.70	75–225	*p*-Alkylaromatic ether	No[52]
Escitalopram (Lexapro®) (**79**) 	18	Antidepressant Generalized anxiety disorder	Base	324	3.13	36.26	10–20	None	N.D.[c]
Enoxaparin (Lovenox)[a]	19	Anticoagulant							
Olanzapine (Zyprexa®) (**94**) 	20	Schizophrenia Bipolar disorder	Base	312	3.00	30.87	10	Benzodiazepine, Thiophene	Yes[7,128]

[a] Biologic. [b] Top end of the daily dose is the maximum recommended dose. [c] N.D., not determined (no published accounts in the primary literature). [d] Requires RM formation for primary pharmacologic activity.

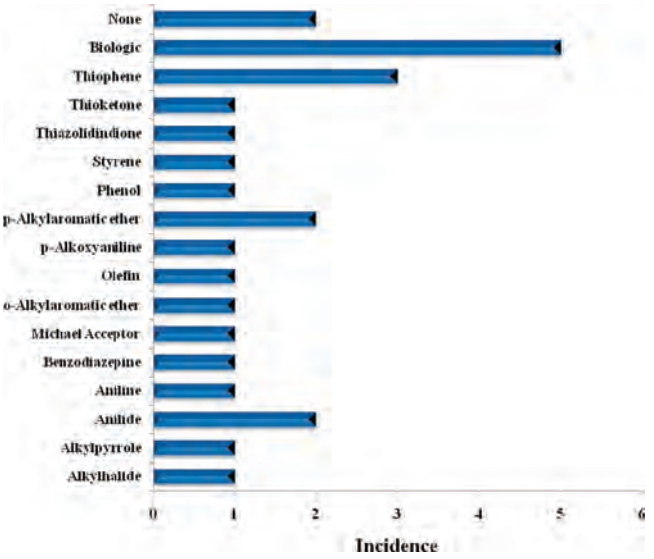


Figure 7. Structural alert analysis in the top 20 drugs in the US market based on 2009 sales.

associated with a high incidence of IADRs despite forming RMs and despite being administered at a relatively high daily dose of 75 mg? A plausible reason is that the majority (>70%) of the clopidogrel daily dose is rapidly hydrolyzed by human carboxylesterases to the inactive carboxylic acid metabolite (~80−85% of circulating metabolites) (Scheme 31),[249] which means that only a small percentage of the parent drug (20 mg or less) is theoretically available for conversion to the active RM. Indeed, covalent binding to platelets accounts for only 2% of radiolabeled clopidogrel in human mass balance studies (Plavix package insert).

The antiasthma drug montelukast (**78**) possesses a styrene motif, which appears to be intrinsically electrophilic, upon the basis of evidence of the direct conjugation of montelukast with a dansylated derivative of GSH in HLM in an NADPH-independent fashion.[242] While these *in vitro* findings raise the possibility of a toxicological outcome via thiol depletion *in vivo*, clinical experience with montelukast has revealed an excellent safety record especially with respect to IADRs. The low daily dose (10 mg) and *in vivo* metabolic fate in humans, which consists of innocuous biotransformation pathways (hydroxylation of the gem-dimethyl groups and *S*-oxidation),[250] serve as plausible explanations for the lack of IADRs despite the propensity to form GSH conjugates.

No evidence for RM formation exists with levothyroxine (**72**), metoprolol (**77**), salbutamol (**80**), and simvastatin (**81**), all of which possess structural alerts. The thyroid hormone levothyroxine (**72**) contains the iodobenzene group which, in principle, can form a putative reactive epoxide in a manner similar to that of bromobenzene; however, the major elimination pathway of this drug involves conjugation of the phenolic OH group to form a sulfate metabolite.[251] The β blocker metoprolol (**77**) contains the *para*-alkylaromatic ether architecture, which upon *O*-dealkylation can yield a quinone-methide precursor. While metoprolol is extensively metabolized in humans by CYP enzymes, the oxidative biotransformation pathways do not feature the proposed bioactivation sequence and instead proceed via *N*-demethylation, benzylic hydroxylation, and *O*-demethylation of the terminal methoxy group.[252] The phenol group in the β 2-agonist

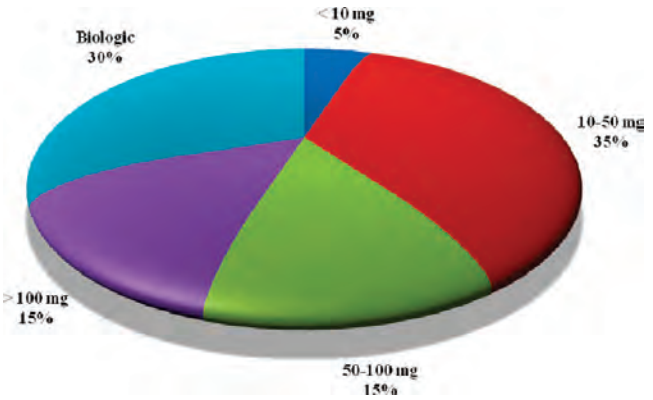


Figure 8. Maximum recommended daily dose assessments for the top 20 drugs in the US market based on 2009 sales.

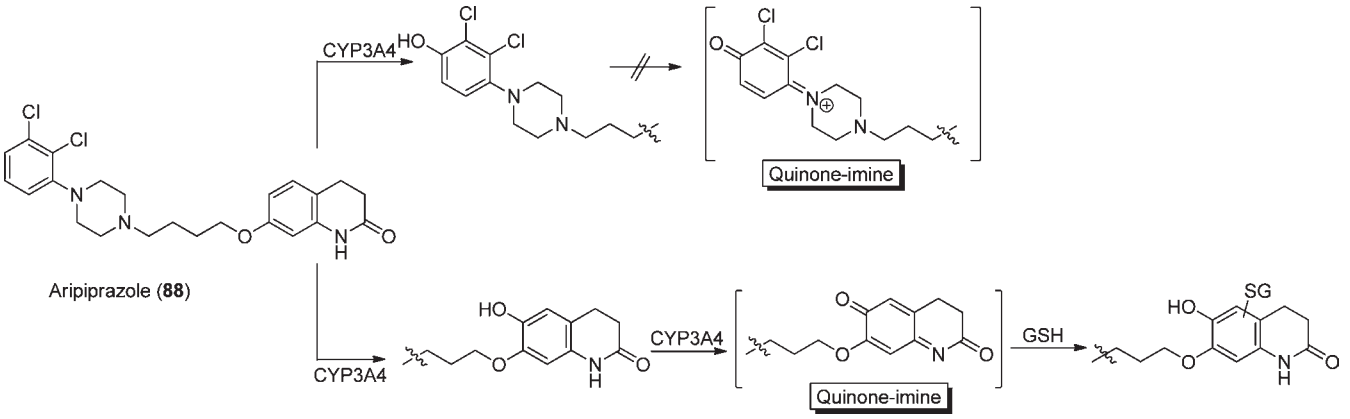
salbutamol (**80**) can be oxidized to an *ortho*-quinone via the catechol intermediate. However, conjugation of the phenol moiety is the principal pathway through which salbutamol is cleared in humans.[253] While the olefin alerts in simvastatin (**81**) are the principal sites of oxidative metabolism by CYP3A4 to the corresponding hydroxy and dihydrodiol metabolites,[254] there is no GSH trapping data to support the involvement of putative epoxide intermediates in the course of simvastatin biotransformation. Weak covalent binding to HLM by simvastatin has been demonstrated;[255,256] the requirement of NADPH further supports the role of CYP3A4 in the bioactivation process. It is also interesting to note that despite BSEP inhibitory effects of simvastatin with potency comparable to known hepatotoxicants (e.g., troglitazone and nefazodone),[93] no evidence for cholestatic injury has been discerned in the clinical use of the statin for over a decade. It is likely that at low clinically efficacious doses of simvastatin, liver concentrations required to inhibit BSEP are not achieved due to the high first pass metabolism of this statin.

6.0. STRUCTURAL ALERT AND RM ANALYSES FOR THE TOP 20 DRUGS IN THE US MARKET IN 2009, BY SALES

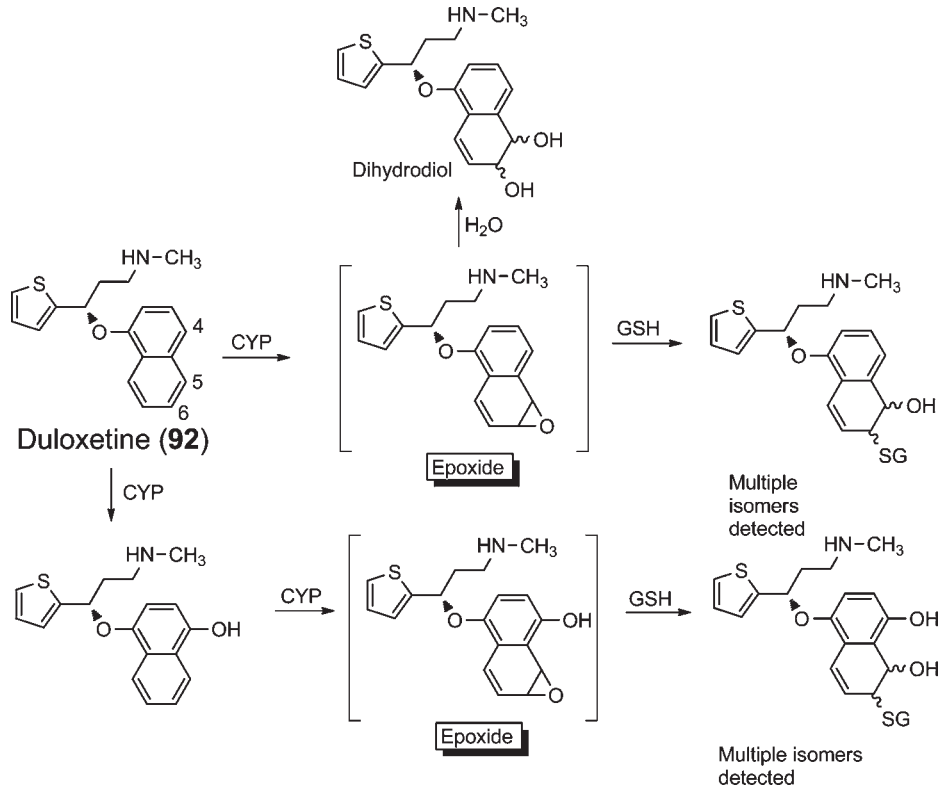
Of the top 20 drugs in the US market by sales (Table 4), six (etanercept, epoetin alfa, inflixmab, bevacizumab, pegfilgrastim, and Lovenox) are biologics, and an additional five drugs (atorvastatin, esomeprazole, clopidogrel, montelukast, and escitalopram) also made the top 20 list of most widely dispensed medicines. Overall, it is interesting to note that 13 out of the 15 small molecule drugs, which constitute the top 20 drugs based on sales, possess structural alerts. The alerts are fairly diverse in nature and include thiophene, aniline/anilide, olefin, and quinone precursors (Figure 7). *In vitro* and/or *in vivo* experimental evidence for RM formation has been presented for 10 out of the 13 drugs. With a few exceptions, the recommended daily doses in this list are fairly low (Figure 8). Three members in this list, namely, quetiapine (**87**), olanzapine (**94**), and pioglitazone (**89**), were discussed earlier.

Fluticasone (**85**) is a synthetic trifluorinated glucocorticoid with potent anti-inflammatory action and is used in patients with asthma, either by itself or in combination with the long-acting β2-adrenoceptor agonist salmeterol (**86**). The principal metabolic fate of fluticasone, which involves an oxidative cleavage of the α-haloalkylthioester motif by CYP3A4/3A5 to the corresponding carboxylic acid metabolite, also leads to a selective mechanism-based

Scheme 32. Bioactivation of Aripiprazole (88) to RMs in HLM



Scheme 33. Bioactivation of the Antidepressant Duloxetine (92) in HLM



inactivation of the CYP3A5 isozyme.[257,258] Details on the bioactivation pathway (especially the structure of the RM) leading to CYP3A inactivation by fluticasone remain unknown. The Michael acceptor structural alert in fluticasone is latent toward metabolism/bioactivation. The low daily dose of fluticasone (0.1 mg), which is delivered directly to the site of action in the lung via inhalation, most certainly mitigates any prospects of IADRs as well as DDIs with CYP3A4/5 substrates including salmeterol, which is also metabolized by CYP3A4.[259] Salmeterol (**86**) is a phenol with propensity to form a reactive quinone through an intermediate catechol metabolite. However, the compound is exclusively metabolized by CYP3A4 on the aliphatic carbon chain possessing the unsubstituted benzene ring to yield α-hydroxysalmeterol, which is then eliminated in the feces.[259]

Since its introduction in 2003, aripiprazole (**87**) has proven beneficial in the treatment of several CNS disorders. Like the hepatotoxicant nefazodone (**48**, Scheme 19), CYP3A4-mediated aromatic hydroxylation on the 2,3-dichlorophenylpiperazine ring in aripiprazole results in the formation of the *para*-hydroxyaripiprazole circulating metabolite, which can form a reactive quinone-imine species similar to nefazodone (Scheme 32). Absence of GSH adducts derived from conjugation with the *para*-hydroxyaripiprazole metabolite in HLM incubations, however, suggests that the bioactivation pathway does not occur with aripiprazole.[158]

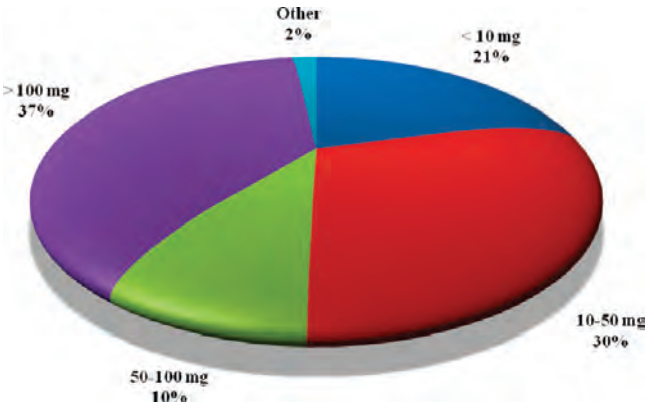


Figure 9. Maximum recommended daily dose assessments for the remaining 180 drugs in the US market by dispensed prescription in 2009.

Besides the arylpiperazine/aniline alert, aripiprazole also contains an acetanilide alert that has been shown to form a reactive quinone-imine intermediate in HLM via the mechanism depicted in Scheme 32. A likely explanation for the markedly improved safety profile of aripiprazole (versus nefazodone), despite the accompanying RM liability, is the vastly improved human pharmacokinetics of aripiprazole (aripiprazole, oral bioavailability = 87%; clearance = 0.8 mL/min/kg, half-life = 75 h; nefazodone, oral bioavailability = 20%, clearance = 7.5 mL/min/kg, half-life = 1 h) due to reduced CYP3A4-mediated metabolism/bioactivation, which translates to a significantly lower daily dose (10−15 mg) compared with that of nefazodone (200−600 mg).[260] The propensity of aripiprazole to inhibit BSEP and/or mitochondrial function has not been published as yet.

Rosuvastatin (**90**) contains an allylic alcohol motif, which can undergo conjugation with GSH. However, *in vivo* disposition studies on rosuvastatin in humans reveal that metabolism is of minimal importance in the clearance of this statin; biliary efflux mediated by transport proteins results in fecal elimination of the majority of rosuvastatin oral dose in the unchanged form.[261]

Duloxetine (**92**) is a potent serotonin and norepinephrine reuptake inhibitor that was approved in 2004 for the treatment of depression and generalized anxiety disorder. Duloxetine contains a pendant thiophene ring, which can be oxidized by CYP enzymes to generate RMs. Indeed, incubation of duloxetine in NADPH- and GSH-supplemented HLM revealed the presence of several GSH conjugates.[262] Interestingly, structural characterization of these conjugates reveal that GSH conjugation occurs on the naphthalene ring rather than on the thiophene structural alert and likely proceeds via a reactive epoxide intermediate (Scheme 33).[262] The involvement of CYP1A2 in duloxetine bioactivation is consistent with the time- and concentration-dependent inhibition of the isozyme by duloxetine, presumably through covalent modification by the electrophilic epoxide species.[263] The bioactivation findings are consistent with the known metabolic pathways of duloxetine in humans that primarily involves oxidation of the naphthyl ring at either the 4-, 5-, or 6-positions followed by further oxidation, methylation, glucuronidation, and sulfation.[264] Despite the RM and CYP inactivation liability, IADRs as well as pharmacokinetic DDIs are extremely rare with this antidepressant, despite an efficacious daily dose of 60 mg.[265]

The antidepressant venlafaxine (**93**) and the opioid analgesic oxycodone (**91**) contain a *para*-alkylaromatic ether scaffold, which can form a reactive quinone methide species upon *O*-demethylation and subsequent two-electron oxidation of the resultant *para*-alkylphenol metabolite. Human mass balance studies indicate venlafaxine *O*-demethylation to the phenol metabolite as a major metabolic fate; however, there is no evidence for oxidation of this metabolite to a reactive quinone-methide species (inferred from the lack of mercapturic acid conjugate(s) in circulation or in human urine).[266] Consistent with the *in vivo* findings, little to no protein covalent binding is discerned in venlafaxine incubations in NADPH-supplemented HLM.[52] *In vivo*, the detection of the corresponding glucuronide and sulfate conjugates of the phenol metabolite of venlafaxine represents a competing metabolic process (relative to bioactivation).[266] Finally, venlafaxine is not a BSEP inhibitor.[93] In the case of oxycodone, there is no data to indicate RM formation by CYP enzymes, and furthermore, the opioid is devoid of detrimental effects on cell viability in human hepatic tissue.[267]

7.0. STRUCTURAL ALERT AND RM ANALYSES FOR THE REMAINING 180 DRUGS IN THE US MARKET IN 2009, BY DISPENSED PRESCRIPTIONS

Table 1 in the Supporting Information depicts structural, physicochemical, and daily dose information for the remainder of the top 200 prescribed drugs. Although lamotrigine (**45**) and sulfamethoxazole (**54**)/trimethoprim (**58**) (Co-trimoxole) carry BBWs, they are still widely prescribed. Besides lamotrigine, sulfamethoxazole/trimethoprim, and acetaminophen (which frequently appears in the list in combination with other analgesics), incidences of mechanism-independent IADRs have been reported only with a handful of drugs on the list (e.g., furosemide (**96**), trazodone (**104**), fluconazole (**127**), amitriptyline (**158**), and niacin (**160**)). Some drugs from the top 20 list based on total sales (albuterol (**80**), fluticasone (**85**), fluticasone (**85**)/salmeterol (**86**) combination, quetiapine (**87**), aripiprazole (**88**), pioglitazone (**89**), rosuvastatin (**90**), duloxetine (**92**), venlafaxine (**93**), and olanzapine (**94**)) also make the list of most widely prescribed drugs for 2009. As stated earlier, multiple ranks for the individual drugs indicate that generic versions are marketed by more than one vendor. There are several instances where a particular drug is repeated due to its combination with other medications. For instance, the loop diuretic hydrochlorothiazide (**95**) appears as a single agent, as well as in combination with antihypertensive agents, lisinopril (**73**), valsartan (**100**), losartan (**128**), triamterene (**141**), and olmesartan (**139**). Likewise, in the "pain-killer" segment, there are multiple entries for acetaminophen (**70**) in combination with analgesics such as oxycodone (**91**), codeine (**126**), and propoxyphen-*N* (**129**). The estrogenic contraceptive ethinyl estradiol (**122**) is sold individually as well as in combination with other steroidal contraceptives including drospirenone (**121**), norgestimate (**163**), and norethindrone (**181**). Finally, the list also includes several biologics, small molecule injectibles, topical agents, vitamins and mineral supplements (e.g., insulin glargine (rank 54), vitamin D (rank 67), potassium chloride (ranks 115 and 192), digoxin (rank 99), latanoprost (rank 120), lidocaine 1% (rank 194), Klor-Con M20, M10 (ranks 147 and 187), and omega 30 acid ethyl esters (rank 191)), which have not been considered in the discussion. Maximum recommended daily doses of the drugs are depicted in Figure 9.

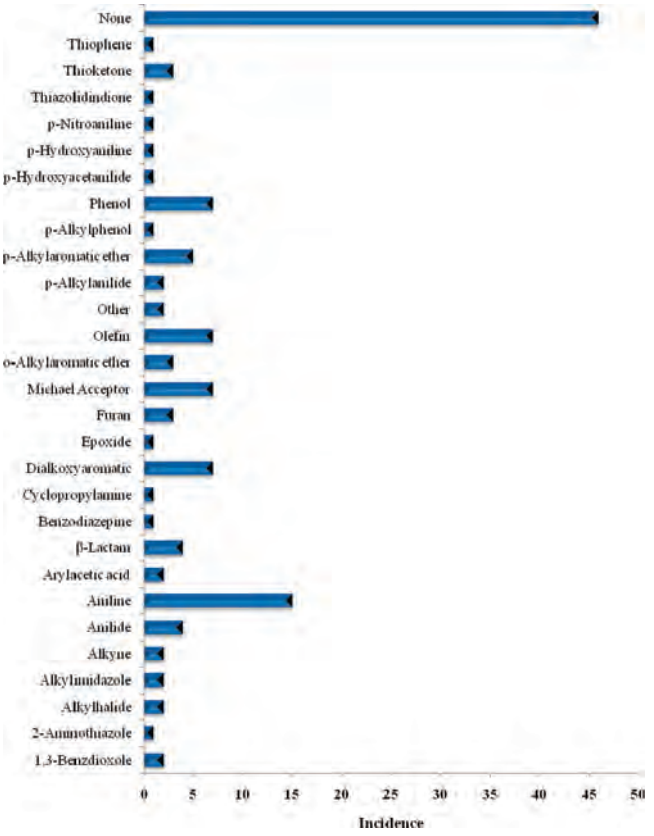


Figure 10. Structural alert analysis in the remaining 180 drugs in the US market by dispensed prescriptions in 2009.

Out of the shortened list of ~93 compounds, 45 drugs are devoid of structural alerts. These compounds include several aliphatics with low (e.g., alendronic acid (**105**), risedronic acid (**145**), and ibandronic acid (**176**)) to high (e.g., gabapentin (**123**) pregabalin (**124**), carisoprodol (**147**), memantine (**149**), isosorbide mononitrate (**169**), etc.) daily doses. Additional drugs that lack structural alerts include warfarin (**97**), zolpidem (**101**), alprazolam (**103**) tramadol (**108**), atenolol (**113**), celecoxib (**116**), valacylcovir (**130**), methylphenidate (**137**), fexofenadine (**138**), citalopram (**150**, the racemic form of escitalopram), sitagliptin (**156**), allopurinol (**161**), lisdexamfetamine (**168**), bupropion (**162**), doxycycline (**172**), eszopiclone (**177**), and meclizine (**180**). There is no description of RM formation, BSEP inhibition, and/or cellular dysfunction in the literature for most of the compounds that are devoid of structural alerts. One can only conclude that lack of *in vitro* safety findings tracks with the general clinical safety, despite administration at high daily doses in several cases (e.g., tramadol at 200−300 mg, celecoxib at 200−400 mg, bupropion at 300 mg, etc.).

Some exceptions to these general observations were also obvious in this analysis. For instance, fenofibrate (**115**) and glyburide (**166**) do not contain structural alerts, but are associated with potent BSEP inhibition.[93] While the finding does not appear to impact fenofibrate safety in the treatment of dyslipidaemia, the antidiabetic agent glyburide is associated with much greater incidence and severity of hepatic injury than any of the older sulfonyl ureas such as chlorpropamide, which is not a BSEP inhibitor.[268] Thus, the potent inhibitory effects of glyburide against BSEP may be a crucial determinant of its hepatic injury

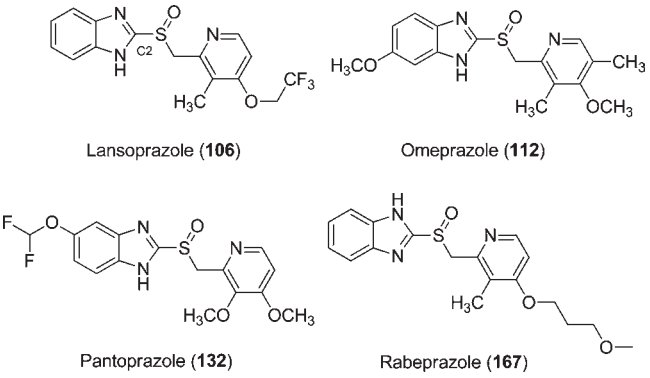


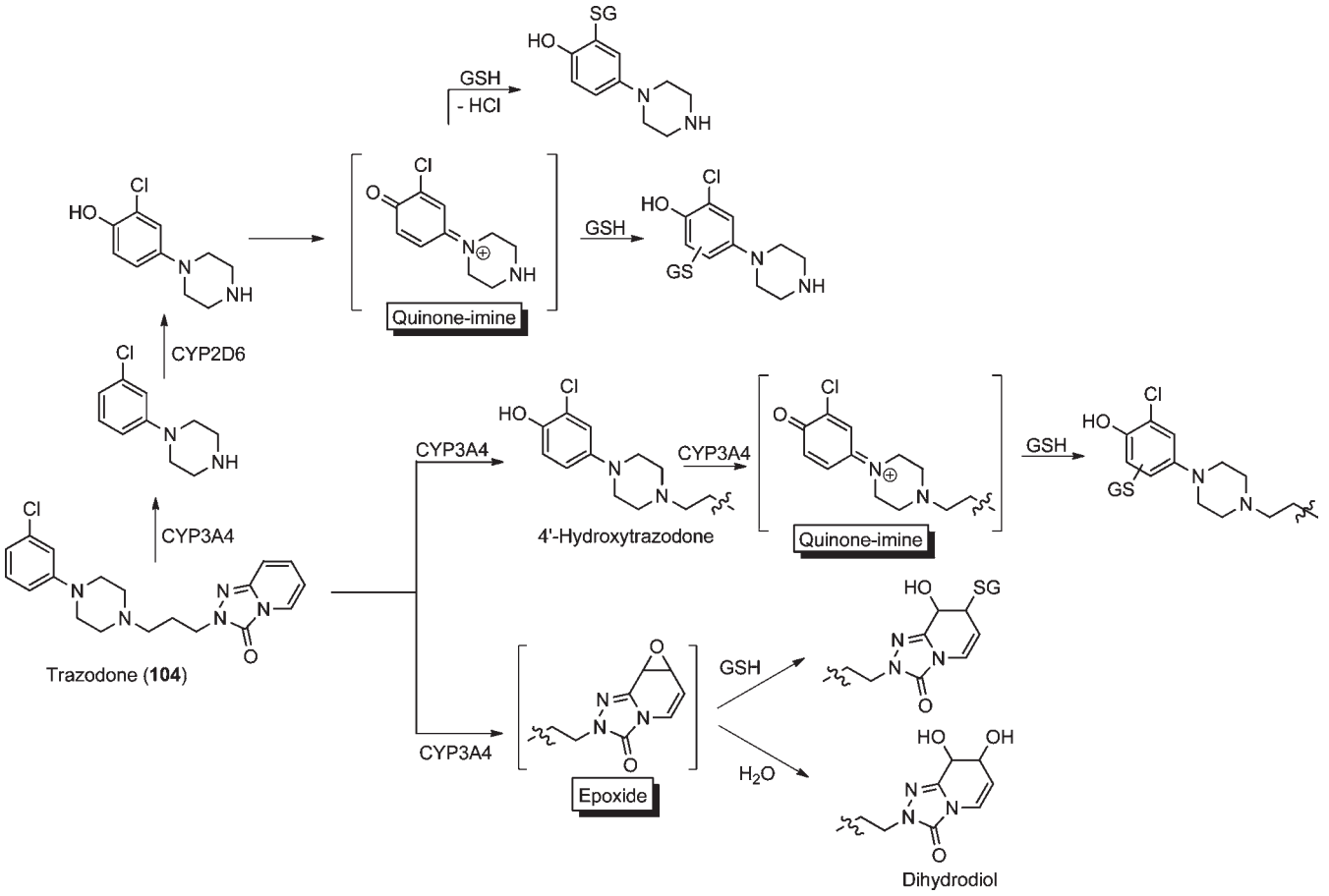
Figure 11. Structures of the proton pump inhibitors that require bioactivation for pharmacologic activity.

potential despite a low daily dose of 20 mg. Likewise, the azole antifungal agent fluconazole (**127**) does not contain alerts in its structure, but its use has been associated with severe or lethal hepatotoxicity.[269] The spectrum of hepatic reactions ranges from mild transient elevations in transaminase levels to hepatitis, cholestasis, and fulminant hepatic failure.[270] Fatal reactions can also occur in patients with serious underlying medical illness.[271] Related azole antifungals ketoconazole and itraconazole are potent BSEP inhibitors, which raises the possibility that hepatotoxicity associated with fluconazole is mediated via the inhibition of the bile salt transporter.[93]

The antihyperlipidemic drug with the highest potential for hepatic injury is the sustained-release formulation of niacin (**160**). The typical pattern of injury involves an elevation in aminotransferase levels, although a mixed pattern of hepatocellular and cholestatic injury has also been discerned.[272] Any formulation of niacin can cause hepatotoxicity in daily doses that exceed 2000−3000 mg, but the sustained-release formulation is significantly more hepatotoxic.[273] The immediate-release formulations of niacin in usual therapeutic doses almost never causes serious liver injury.[274] Although niacin does not contain conventional structural alerts, the hepatotoxic effects are believed to be related to its metabolism by a high-affinity, low-capacity amidation pathway that leads to nicotinamide (niacinamide), and *N*-methyl-2- and *N*-methyl-4-pyridone-5-carboxamide metabolites; thus, the sustained release formulation can lead to higher levels of toxic metabolites.[275] The alternative competing metabolic pathway is a low-affinity, high-capacity conjugation pathway (involving the formation of a glycine amide metabolite) that leads to prostaglandin-mediated vasodilation and subsequent cutaneous flushing.[274] The immediate release formulation overwhelms the higher affinity amidation/oxidation pathway, and the majority of the niacin dose is metabolized via the high-capacity glycine conjugation pathway, leading to a much lower rate of hepatotoxicity.[275] Extended-release niacin has an intermediate rate of dissolution and can be associated with both flushing and hepatotoxicity.

Of the 48 drugs that contain structural alerts (Figure 10), evidence for RM formation has been presented in ~19 (39%) cases. Foremost among these are prescription strength NSAIDs ibuprofen (**98**) and naproxen (**159**) that form electrophilic acyl glucuronides as a principal metabolic fate in humans.[36,276] IADRs such as hepatotoxicity are extremely rare with these NSAIDs despite administration at very high doses.[36] Like ibuprofen, the reactivity and covalent binding potential of naproxen glucuronide with nucleophiles and albumin has been demonstrated.[277,278]

Scheme 34. Bioactivation of the Antidepressant Trazodone (104) in HLM



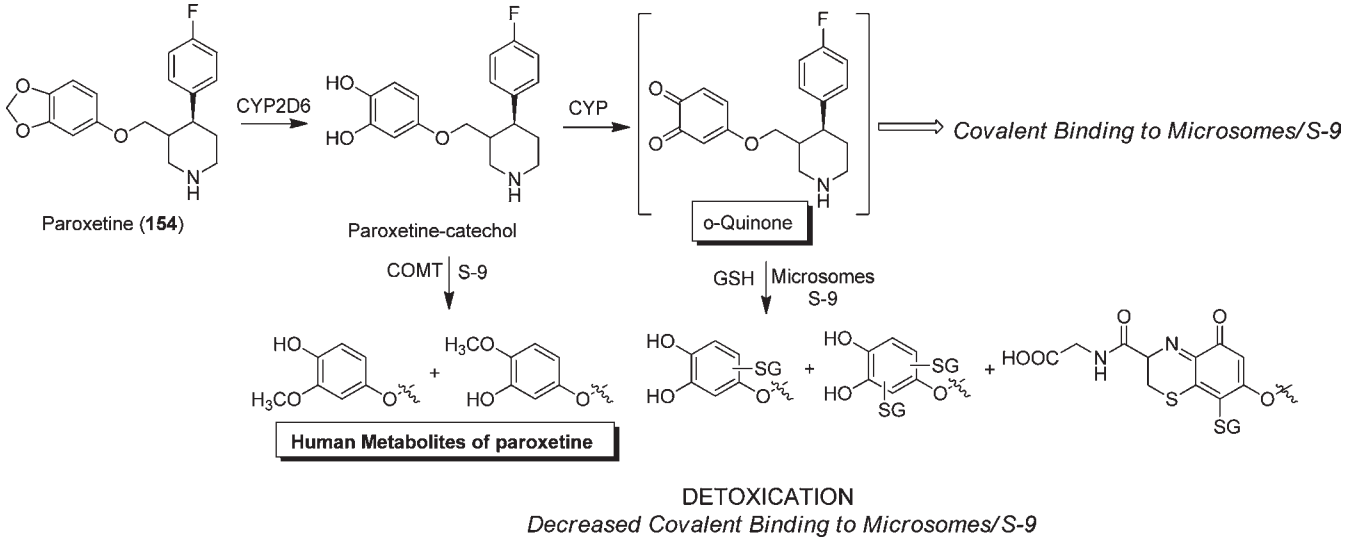
Penicillin VK (**164**) is a β-lactam antibacterial that irreversibly acylates the active site serine of serine-type carboxypeptidase forming a serine ester linked adduct in a manner similar to that of amoxicillin (**74**).

The list of RM positives also includes lansoprazole (**106**), omeprazole (**112**, the racemic form of esomeprazole), pantoprazole (**132**), and rabeprazole (**167**), which require bioactivation for their pharmacologic action similar to that of esomeprazole (**75**) (Figure 11). In addition, GSH conjugates derived from the reaction of the thiol at the C2 position of the benzimidazole ring have been observed in HLM and in rats, phenomena that reflect the electrophilic nature of the benzimidazole-*S*-oxide motif in these compounds.[242,243,279] Despite the RM liability, these drugs are widely prescribed and are generally devoid of IADRs. Like esomeprazole, the generic proton pump inhibitors are low daily dose drugs. Examination of BSEP inhibition by omeprazole and lansoprazole reveal no inhibitory effects.[93]

Potassium clavulanate (**114**) is used in conjunction with amoxicillin (**74**) (combination sold as augmentin) to destroy penicillin-resistant strains of bacteria. By itself, clavulanate does not possess antibacterial activity. Bacteria produce the serine hydrolase β-lactamase to hydrolyze carboxypeptidase inhibitors, forming a hydrolytically labile serine-ester linked adduct.[280] Reactions between the catalytic serine residue in β-lactamase and specific β-lactamase inhibitors such as clavulanate (**114**) result in the formation of stable adducts derived from β-lactam ring-opening, functionally inactivating the bacterial resistance mechanism.[280] While the rate of hepatic injury with amoxicillin is very low, combination with clavulanate increases this risk.[235] Evidence that clavulanate is principally responsible for hepatotoxicity stems from the observation that clavulanate combined with other β-lactams can also lead to liver injury.[281] A genetic basis for amoxicillin/clavulanate hepatotoxicity has been identified with linkage to the HLA haplotype suggesting that the IADR has a immunologic component.[282] Furthermore, individuals with glutathione transferase null genotypes are known to be at an increased risk of amoxicillin−clavulanate hepatotoxicity, a finding that is consistent with a deficiency in clavulanate detoxication by GSH.[283] Mechanistic studies on the chemical and/or biochemical reactivity of clavulanate with GSH and related protein nucleophiles may shed light on the pharmacogenomic findings.

Furosemide (**96**) is a potent loop diuretic used in the treatment of edematous states associated with cardiac, renal, and hepatic failure, and for the treatment of hypertension. Furosemide use has been associated with cases of idiosyncratic hepatitis, which have been linked with the bioactivation of its furan substituent to a reactive epoxide species by CYP enzymes.[129,284−286] Similar to acetaminophen, furosemide hepatotoxicity is dose-dependent and can be replicated in rodents.[286] Although the NADPH-dependent microsomal covalent binding of furosemide is significantly attenuated in the presence of GSH,[129] the toxicity of furosemide in mice does not result from simple GSH depletion as is the case with acetaminophen.[286] There is no evidence of BSEP inhibitory effects[93] or mitochondrial injury with furosemide.[287]

Scheme 35. Competing Bioactivation/Detoxication Pathways of Paroxetine (154) in Human Hepatic Tissue[a]



[a] Plausible explanation for the lack of IADRs despite RM formation.

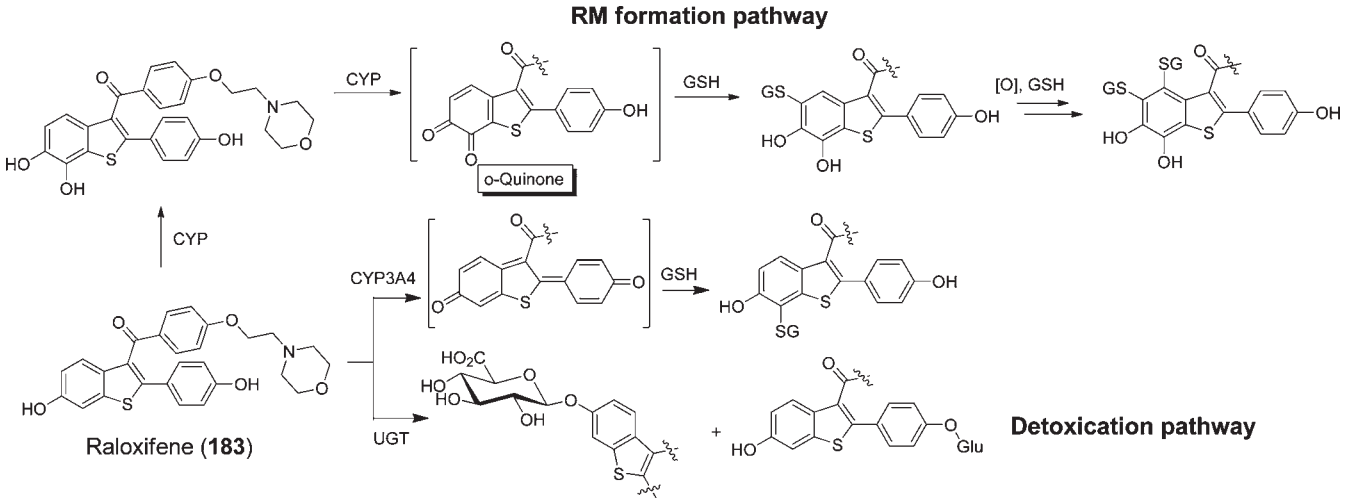
Trazodone (**104**) is a second generation nontricyclic antidepressant, which is often coprescribed with other antidepressants as a sleep-inducing agent because of its sedative side effects. Although trazodone has not served as frontline therapy in the treatment of CNS disorders in a manner similar to that of nefazodone, there are some reported cases of rare but severe hepatotoxicity associated with trazodone use,[288,289] which have been linked to RM formation (Scheme 34).[290,291] Like nefazodone (**48**), the 3-chlorophenylpiperazine ring in trazodone is oxidatively metabolized by CYP3A4 to *para*-hydroxytrazodone, which has been detected as a major urinary metabolite in humans.[292,293] A reactive quinone-imine species that is formed via the two electron oxidation of *para*-hydroxytrazodone has been trapped with GSH in HLM incubations of trazodone.[290] An identical bioactivation phenomenon has also been demonstrated with 3-chlorophenylpiperazine, the *N*-dealkylated metabolite of trazodone.[291] In addition to 3-chlorophenylpiperazine ring bioactivation, the detection of a dihydrodiol metabolite of trazodone in human urine suggests a second bioactivation sequence involving the formation of an electrophilic epoxide on the triazolopyridinone moiety in trazodone.[292,293] Consistent with this hypothesis, HLM and recombinant CYP3A4 incubations of trazodone in the presence of GSH has led to the detection of stable dihydrodiol and a GSH conjugate derived from epoxide ring-opening.[290] While the bioactivation pattern of trazodone resembles that seen with nefazodone to some degree, it is interesting to note that trazodone does not possess deleterious effects on mitochondrial function and BSEP activity in a manner similar to that of nefazodone.[160,161]

Paroxetine (**154**) is a selective serotonin reuptake inhibitor, which contains the 1,3-benzdioxole structural alert. The principal pathway of paroxetine metabolism in humans involves CYP2D6-mediated 1,3-benzdioxole ring scission to a catechol intermediate. This process also leads to mechanism-based inactivation of the CYP isozyme and DDIs with CYP2D6 substrates in the clinic.[294] *In vitro* studies with [^3H]-paroxetine have demonstrated the NADPH-dependent covalent binding to human microsomal and S9 proteins, and the characterization of GSH conjugates of reactive quinone metabolites (Scheme 35).[295] The observation that NADPH-dependent protein covalent binding in HLM or human S9 fraction is dramatically reduced in the presence of GSH, or *S*-adenosyl methionine implies the existence of competing, detoxication pathways of paroxetine metabolism.[295] As shown in Scheme 35, the catechol metabolite obtained via ring scission of the 1,3-benzdioxole group in paroxetine can partition between *O*-methylation by COMT or undergo oxidation to the reactive *ortho*-quinone intermediate, which is scavenged by GSH; both pathways lead to a significant reduction in covalent binding. In humans, the *O*-methylated catechol derivatives constitute the principal metabolic fate of the drug.[296] When coupled with the fact that the clinically effective daily dose of paroxetine is low (20 mg), some insight into the excellent safety record of this drug is obtained, despite the RM liability. Sertraline (**99**) and fluoxetine (**110**) are widely prescribed selective serotonin reuptake inhibitors, which are devoid of structural alerts. While fluoxetine is not prone to RM formation, sertraline is known to exhibit weak microsomal covalent binding in a NADPH-dependent fashion.[52] Although the biochemical basis for the NADPH-dependent covalent binding by sertraline is unknown, it is important to note that IADRs are extremely rare with this drug at its recommended daily doses of 50−200 mg. While the BSEP inhibitory potential of paroxetine and sertraline is not known, fluoxetine has been evaluated against BSEP and is devoid of inhibitory effects.[93]

The 1,3-benzdioxole alert in tadalafil (**155**) also succumbs to CYP3A4-catalyzed bioactivation generating an electrophilic catechol/*ortho*-quinone species and concomitant enzyme inactivation.[297] However, there are no reports of idiosyncratic toxicity and/or DDIs associated with tadalafil in erectile dysfunction treatment at the recommended low daily doses between 5−20 mg. Against this backdrop, sildenafil (**125**), used for the same indication, does not possess a structural alert.

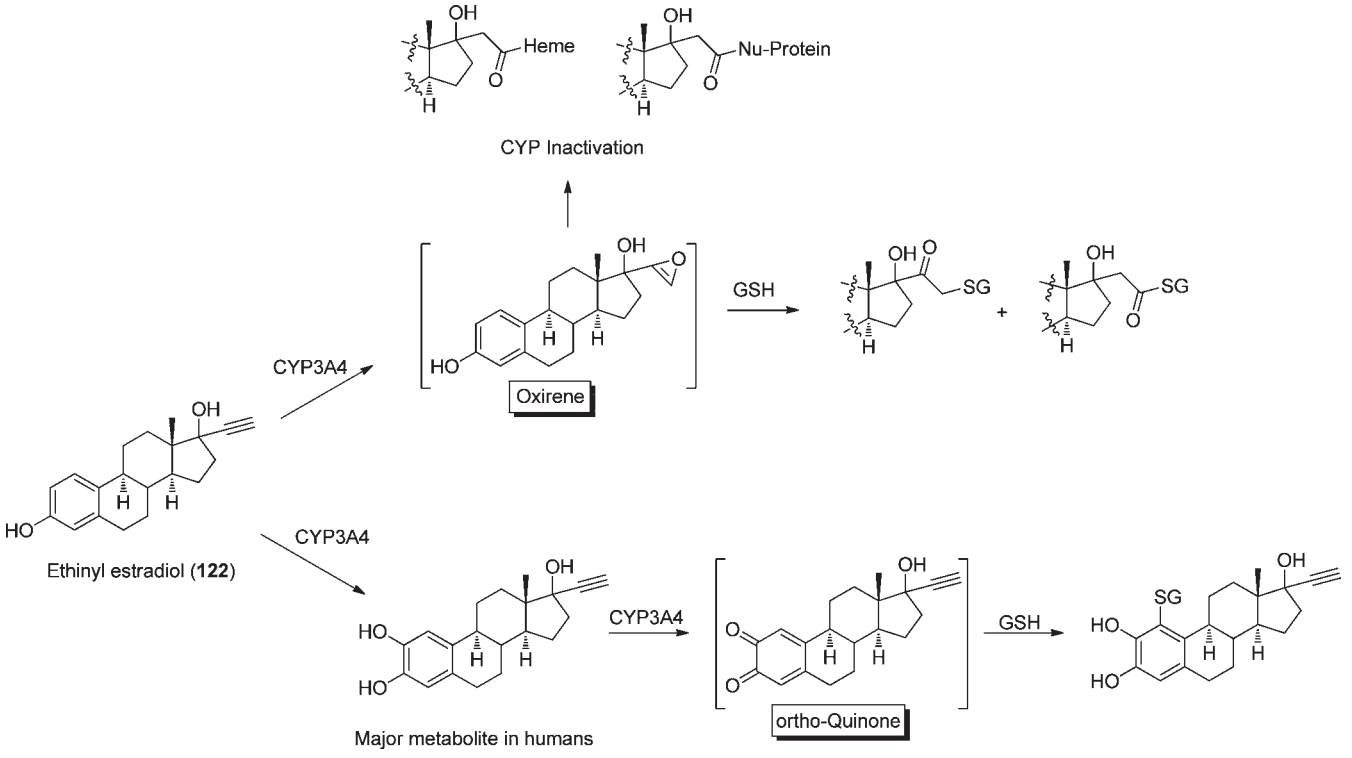
The mixed α- and β-adrenergic receptor antagonist and antihypertensive drug carvedilol (**153**) possesses an aniline and a dialkoxyaromatic functionality that can form RMs. In humans, carvedilol is metabolized through *N*-glucuronidation and CYP2D6-mediated oxidative biotransformation pathways resulting in over 50 metabolites.[298,299] In HLM incubations of carvedilol, GSH

Scheme 36. Competing Bioactivation/Detoxication Pathways of Raloxifene (183) in Human Hepatic Tissue[a]



[a] Plausible explanation for the lack of IADRs despite RM formation.

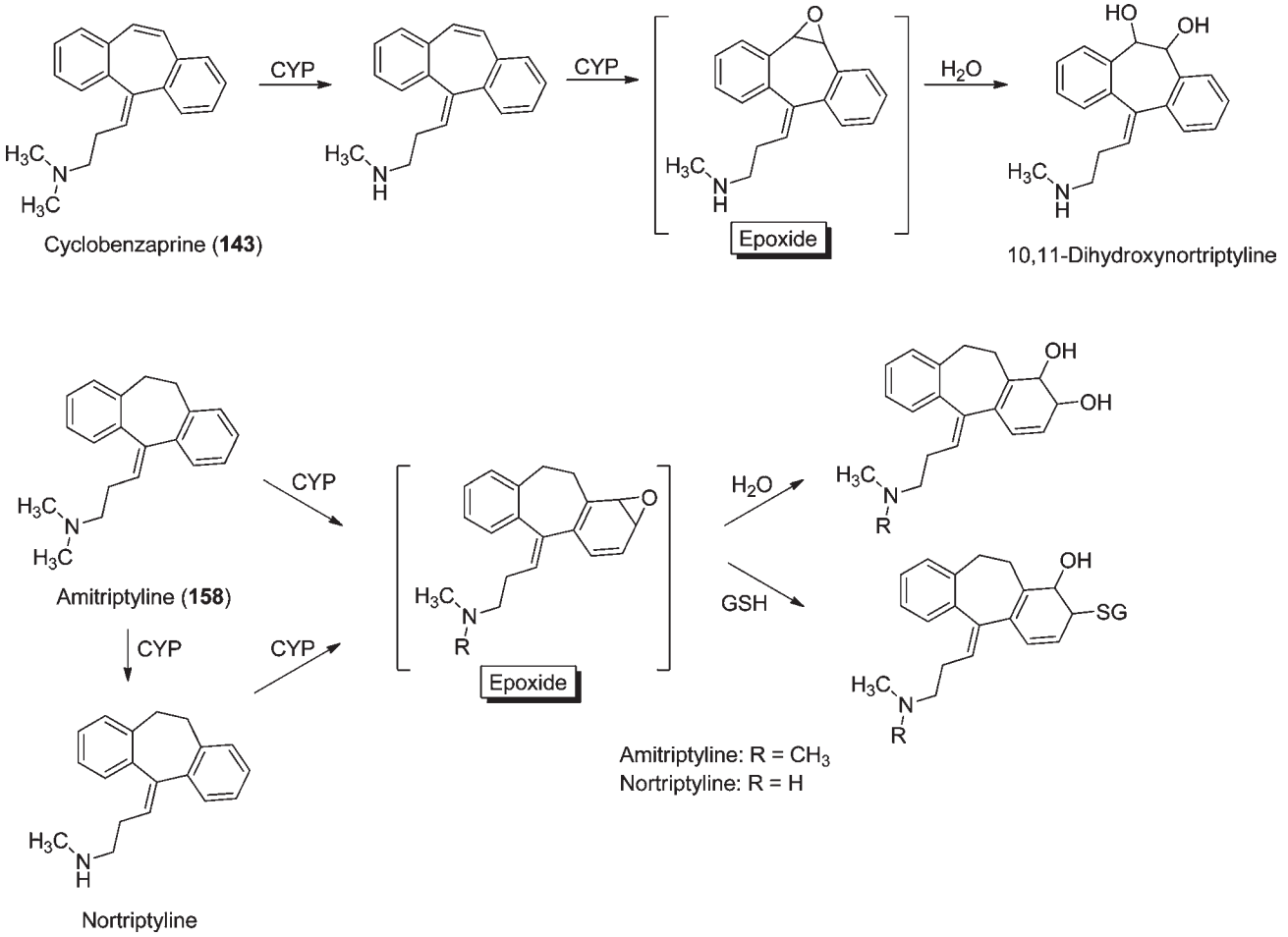
Scheme 37. Bioactivation of the Steroidal Contraceptive Ethinyl Estradiol (122) by CYP3A4 to RMs



conjugates believed to arise from the conjugation of the thiol nucleophile to quinone-imine intermediates and catechol/*ortho*-quinone species have been reported.[299] Formation of the quinone-imine species appears to be compatible with the biotransformation pathway of carvedilol that involves aromatic hydroxylation on the carbazole ring to hydroxyaniline type metabolites.[298] The clinical dose of carvedilol ranges from 6.25 mg to the maximum recommended daily dose of 100 mg and may provide a rationale for the very rare occurrence of IADRs[300] particularly at the low doses used to treat hypertension.

Raloxifene (**183**) is a selective estrogen receptor modulator that is used in the treatment of osteoporosis in postmenopausal women. Raloxifene is known to undergo *in vitro* CYP3A4-catalyzed bioactivation on the phenolic structural alerts to yield reactive quinone species that can be trapped with GSH (Scheme 36).[301−303] The process is also accompanied by microsomal covalent binding and irreversible inactivation of CYP3A4.[302,303] However, *in vivo*, glucuronidation of the same phenolic groups, principally in the gut, constitutes the principal elimination mechanism of raloxifene in humans (Scheme 36).[303,304] Thus, the likelihood of raloxifene

Scheme 38. Oxidative Bioactivation of the Skeletal Muscle Relaxant Cyclobenzaprine (143) and the Structurally Related Tricyclic Antidepressant Amitriptyline (158) in Humans



bioactivation *in vivo* is in question when compared with the efficiency of the glucuronidation in the small intestine, a phenomenon that may provide an explanation for the extremely rare occurrence of IADRs despite a relatively high daily dose of 60 mg.

The estrogenic contraceptive ethinyl estradiol (**122**) is sold individually or in combination with other steroidal contraceptives such as drospirenone (**121**), norgestimate (**163**), and norethindrone (**181**). CYP-catalyzed metabolism of the C-17α terminal alkyne alert in these steroids generates a reactive oxirene species, which alkylates the heme group and/or protein resulting in the mechanism-based inactivation of the isozyme (Scheme 37).[305−310] In addition to acetylene bioactivation, Park et al.[310] have demonstrated the oxidation of the phenol ring in ethinyl estradiol to an electrophilic *ortho*-quinone species (via the intermediate catechol metabolite) in HLM, a process that eventually leads to microsomal covalent binding.[92] Lack of toxicity or DDIs with CYP3A4 substrates is most likely due to the very low doses of these drugs used in birth control. Potent BSEP inhibition also has been established with ethinyl estradiol.[93]

Cyclobenzaprine (**143**) is a skeletal muscle relaxant that contains two olefin structural alerts. While the principal metabolic fate of this drug in humans involves *N*-glucuronidation, a significant proportion (∼7−10% of the administered dose) of a dihydrodiol (10,11-dihydroxynortriptyline) metabolite has been detected in human urine (Scheme 38).[311] The formation of the dihydrodiol metabolite is consistent with olefin epoxidation as a rate-limiting step. Despite the potential RM liability, no IADRs have been reported with cyclobenzaprine at its low daily dose range of 10−30 mg. In contrast, amitriptyline (**158**), the saturated analogue of cyclobenzaprine, has been associated with very rare but severe incidences of hepatotoxicity in the clinic.[312,313] Amitriptyline, along with other tricyclic antidepressants, has been the cornerstone of antidepressant therapy for over three decades. Despite current treatment guidelines that recommend the use of tricyclic antidepressants only in patients with psychosis and treatment resistance, amitriptyline is widely prescribed in the US and throughout the world presumably because of its favorable cost/benefit ratio. A causal link between toxicity and amitriptyline metabolism has been established.[314] In the many biotransformation pathways of amitriptyline in humans, the characterization of a dihydodiol metabolite in urine has been an area of focus.[315,316] Wen et al.[314] have intercepted the intermediate aromatic ring epoxide via GSH trapping studies in HLM (Scheme 38). A similar bioactivation pathway has been noted with nortriptyline, an antidepressant that is also associated with idiosyncratic hepatotoxicity.[317] Nortriptyline is the *N*-dealkylated metabolite of amitriptyline.[315] The reasons for the differences in overall toxicity profiles of cyclobenzaprine and amitriptyline are not apparent, especially since both compounds are capable of generating an electrophilic epoxide intermediate. It is possible that

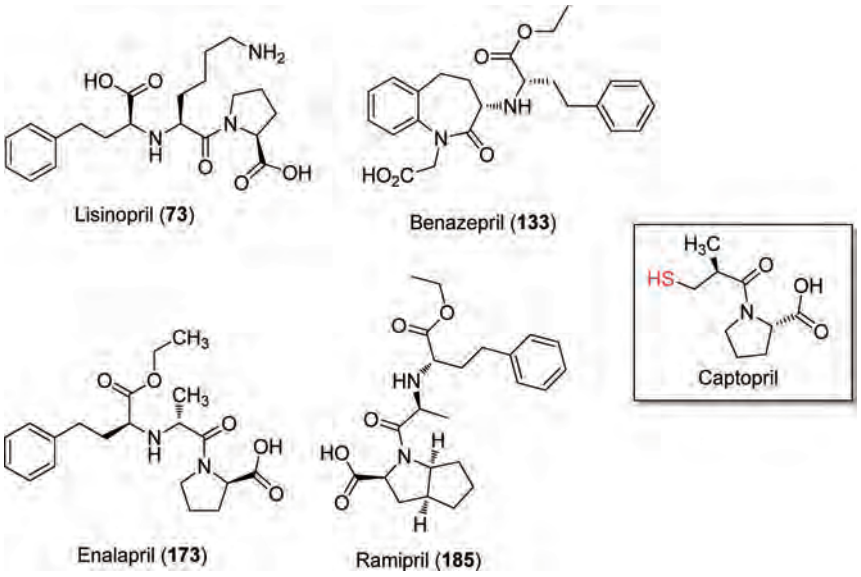


Figure 12. Structure−toxicity relationships of angiotensin-converting enzyme inhibitors.

the manifestation of idiosyncratic toxicity due to RM formation may be related to the higher daily dose of amitriptyline (50−150 mg). Amitriptyline is devoid of BSEP inhibitory effects.[93]

At its recommended daily doses (2−8 mg), buprenorphine (**182**) is safely used as a substitution drug in heroin addicts. However, large overdoses or intravenous misuse has been known to cause hepatotoxicity.[318] NADPH-dependent microsomal covalent binding of buprenorphine and its *N*-dealkylated metabolite norbuprenophine has been demonstrated.[319,320] The identity of the RM responsible for covalent binding is unclear. In addition to the bioactivation phenomenon, buprenorphine was shown to impair mitochondrial respiration and ATP formation.[320]

The loop diuretic hydrochlorothiazide (**95**) is usually marketed individually or in combination with the angiotensin II receptor antagonists valsartan (**100**), losartan (**128**), olmesartan (**139**), or irbesartan (**174**) for the treatment of hypertension. Among these drugs, hydrochlorothiazide, losartan, and olmesartan possess structural alerts. With the exception of losartan, there is no evidence to indicate RM formation with hydrochlorothiazide and olmesartan. Hydrochlorothiazide is principally eliminated in humans via renal excretion in the unchanged form, which is facilitated by organic anion transporters.[321] Olmesartan is also eliminated in urine and feces through active hepatobiliary and renal efflux mediated by transport proteins.[322] In the case of losartan, formation of sulfydryl conjugates and covalent binding has been demonstrated in HLM.[52] The MW of the sulfydryl conjugates suggests that a two-electron oxidation of the alkylimidazole motif in losartan gives rise to a reactive imine-methide species. However, in humans, there is no evidence for RM formation; oxidative metabolism of the primary alcohol moiety by CYP leads to an active carboxylic acid metabolite (∼15% of the administered losartan dose).[323] In addition, the majority of a losartan dose is eliminated in the feces in parent form and as the tetrazole glucuronide conjugate.[323] In contrast with losartan, olmesartan and valsartan do not exhibit HLM covalent binding.[92] While the propensity to inhibit BSEP by valsartan, losartan, olmesartan, and irbesartan is unknown, telmisartan, a structurally related angiotensin II receptor antagonist, is a potent BSEP inhibitor.[93]

Lisinopril (**73**), benazepril (**133**), enalapril (**173**), and ramipril (**185**) (Figure 12) are angiotensin-converting enzyme inhibitors and are used in the treatment of hypertension and/or stroke. Benazepril is the only drug in this group that contains a structural alert (an anilide functionality). However, in humans, benazepril is principally metabolized through ester hydrolysis followed by glucuronidation. There is no involvement of an oxidative metabolic process in the clearance of the drug.[324] While IADRs associated with the use of these four drugs are extremely rare, it is interesting to contrast these observations with captopril, the first marketed ACE inhibitor, which contains a free thiol group (Figure 12). Unlike the recent angiotensin-converting inhibitors, captopril is a very high dose drug. When first marketed, captopril was administered at doses up to 1000 mg in severely hypertensive patients. A series of systemic ADRs including skin rashes, blood dyscrasias, and autoimmune syndromes were reported, and the dose dependency of these effects was observed across studies.[325] Conjugates of the free thiol group in captopril with GSH and with cellular proteins have been observed and have implicated the bioactivation of this SH functionality as a plausible cause for toxicity.[326,327]

Like simvastatin (**81**), lovastatin (**170**) and pravastatin (**140**) are low daily dose drugs (10−20 mg effective dose range) and relatively safe with over a decade of clinical experience. Metabolism of lovastatin (**170**) by CYP3A4 occurs primarily on the 6′-position of the hexahydronaphthalene motif to afford 6′-β-hydroxylovastatin and a potentially electrophilic 6′-exomethylene derivative.[328] GSH trapping and/or HLM covalent binding data on lovastatin have not been reported in the literature. Considering the structural similarity with simvastatin (**81**), it is likely that lovastatin may also demonstrate potent inhibition of BSEP. While renal excretion in the unchanged form is a major route of clearance of pravastatin (**140**), a small percentage of the drug appears to be metabolized by CYP-dependent and -independent pathways to yield monohydroxylated metabolites on the hexahydronaphthalene motif.[329,330] As such, minimal covalent binding has been discerned in NADPH-supplemented HLM incubations of pravastatin.[92] The selective cholesterol absorption inhibitor ezetimibe (**118**) contains phenol and an aniline

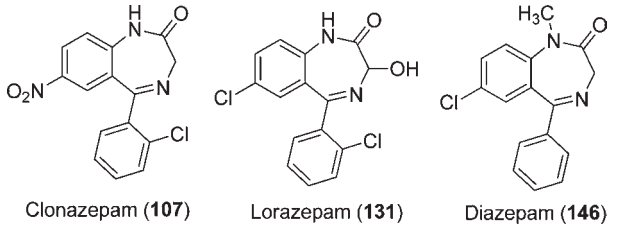


Figure 13. Structures of benzodiazepine-based drugs.

structural alert, which could lead to quinone species upon oxidative metabolism. However, the primary route of elimination of this agent in humans is via phenol glucuroniation with virtually no contribution from CYP enzymes.[331]

In contrast with trovafloxacin, the fluoroquinolone antibiotics, levofloxacin (**109**) and ciprofloxacin (**157**), are devoid of idiosyncratic hepatotoxicity despite administration at high daily doses. From a structure−toxicity standpoint, they do not contain the pendant cyclopropylamine functionality at the C-7 position of the fluoroquinolone scaffold and cannot form RMs in a manner similar to that proposed for trovafloxacin. Both levofloxacin and ciprofloxacin contain aniline structural alerts, and in addition, ciprofloxacin also possesses a cyclopropyl group attached to the quinolone nitrogen atom. However, the major clearance pathways in humans occur via carboxylic acid glucuronidation and/or renal excretion in unchanged form, with little to no contribution from CYP enzymes.[332,333] Consistent with these *in vivo* observations, levofloxacin does not exhibit covalent binding to HLM.[92] The two fluoroquinolones are also devoid of BSEP inhibitory effects.[93]

All benzodiazepine drugs, clonazepam (**107**), lorazepam (**131**), and diazepam (**146**) (Figure 13), contain the aniline structural alert. In addition, clonazepam also contains a *para*-nitroanilide motif. In humans, diazepam and lorzepam are metabolized through innocuous biotransformation pathways (e.g., *N*-demethylation, aliphatic hydroxylation, *O*-glucuronidation, etc.) and do not involve aniline ring metabolism.[334,335] In contrast, the major route of clonazepam metabolism in humans involves the reduction of the *para*-nitroanilide motif to the 7-aminoclonazepam metabolite (presumably via the electrophilic nitroso intermediate), which is then acetylated by polymorphic NAT2.[336,337] There are no literature reports on the formation/trapping of RMs (e.g., nitroso and *bis*-imine intermediates) in the course of clonazepam metabolism. While the clonazepam biotransformation pathway is analogous to that observed with the idiosyncratic hepatotoxicant tolcapone, there is very little evidence for IADRs with clonazepine. It could be suggested that the low daily dose range of 1.5−20 mg mitigates toxicity risks due to RM formation with clonazepam.

Donepezil (**117**) contains the *ortho*-dimethoxyphenyl functionality, which upon sequential *O*-dealkylation can yield a catechol intermediate. While *O*-demethylation is observed as a metabolic fate in humans, the resulting products are efficiently glucuronidated, and there is no evidence for additional *O*-demethylation and/or aromatic ring hydroxylation that can lead to the formation of electrophilic quinones.[338] *O*-Demethylation is also observed as a metabolic fate with verapamil (**186**), a calcium channel blocker, which contains two *ortho*-dimethoxyphenyl substituents. However, like donepezil, the corresponding metabolites are principally eliminated as glucuronides in humans.[339] Verapamil does not exhibit BSEP inhibition.[93] The

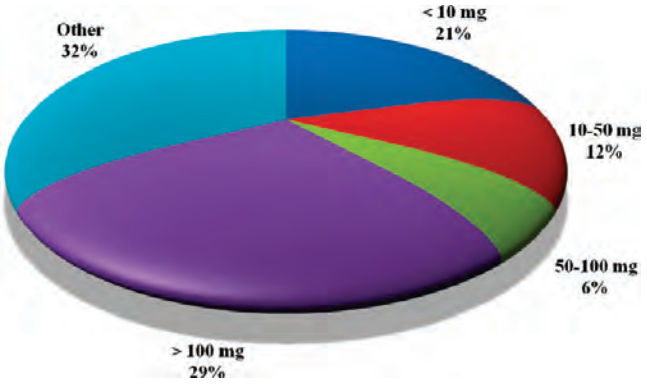


Figure 14. Maximum recommended daily dose assessments in the remaining 180 drugs in the US market based on total sales in 2009.

dialkoxyaromatic motif in the selective α1-adrenoceptor blocker tamsulosin (**102**) also undergoes *O*-dealkylation; however, the corresponding phenolic metabolites are efficiently glucuronidated and sulfated.[340]

Oseltamivir (**135**) contains a cyclic Michael acceptor motif, and despite administration at a fairly high daily dose of 150 mg, the drug is rarely associated with idiosyncratic toxicity. Oseltamivir is a prodrug and requires enzymatic hydrolysis of its ester linkage to afford the active carboxylic acid metabolite. This metabolic step accounts for >80% metabolism of the drug; no conjugates derived from the addition of GSH to the Michael acceptor functionality in either prodrug or active carboxylic acid form have been described. Furthermore, the carboxylic acid metabolite of oseltamivir is eliminated unchanged via renal excretion.[341] Similar to oseltamivir, prednisolone (**111**) and methylprednisolone (**142**) contain a potential Michael acceptor in the A ring of the steroidal architecture. However, in humans, both compounds are metabolized via the reduction of the C-20 carbonyl group, oxidation of the C20−C21 side chain to carboxylic acid metabolites, and hydroxylation at the 6β-position on the B ring.[342] In addition to the A ring quinone motif, furan and alkylhalide alerts are also discerned in the chemical structure of Mometasone (**120**), a glucocorticoid used in the treatment of topical dermatological disorders and allergic rhinitis. Recently, Mometasone has been approved as an oral inhalation powder, in the treatment of mild-to-moderate persistent asthma. There is very limited information on the pharmacokinetics of Mometasone after oral inhalation at the efficacious dose of 0.2 mg. It appears that 6β-hydroxylation is a key metabolic fate with no detectable metabolism on the quinone, furan, and/or alkylhalide functionalitites.[343]

The furan structural alert in the H2 antagonist ranitidine (**187**) does not appear to be problematic from a toxicological standpoint, despite the requirement of a high daily dose of 300 mg. This is because the furan group is latent toward metabolism in ranitidine. Approximately 65−70% of the clearance mechanism in humans involves urinary excretion mediated through transport proteins. Hepatic metabolism (*S*- and *N*-oxidation) appears to contribute little to the overall clearance process.[344] Likewise, the principle clearance mechanism of the inhaled bronchodilator tiotropium in humans involves renal excretion. Potential toxicity risks due to pendant thiophenes and epoxide motif do not appear to be a cause for concern especially when considering a low inhalation dose of ∼18 μg per day.[345]

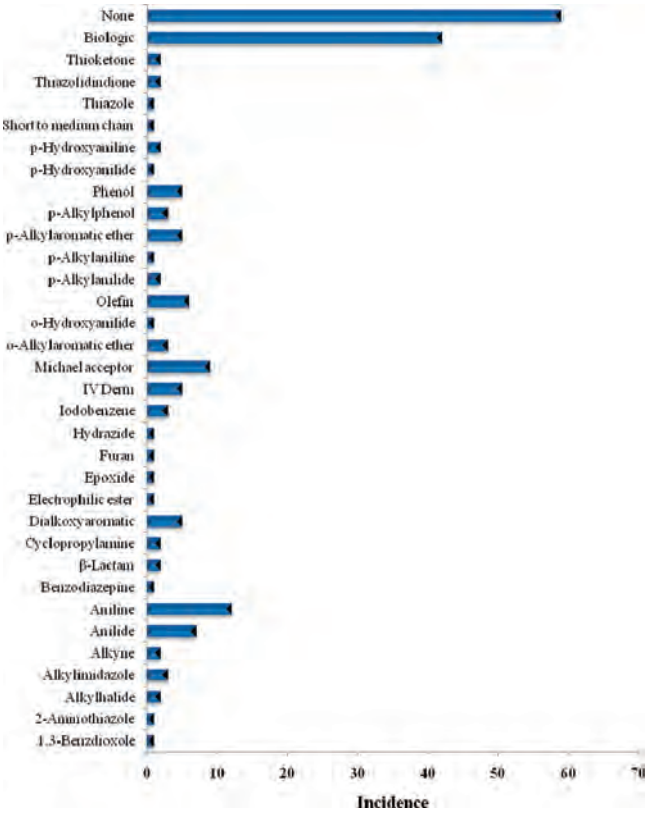


Figure 15. Structural alert analysis in the remaining 180 drugs in the US market based on 2009 sales.

8.0. STRUCTURAL ALERT AND RM ANALYSES FOR THE REMAINING 180 DRUGS IN THE US MARKET IN 2009, BY SALES

Table 2 in the Supporting Information depicts structural, physicochemical, and daily dose information for the remainder of the top 200 drugs, upon the basis of 2009 sales. Maximum daily doses for this list of drugs are shown in Figure 14. In this list of 180 entities, ~39 are biologics, and 19 are small molecule injectibles, dietary supplements, topical, and/or contrast agents. A significant number of the "most prescribed" drugs (>60) in individual form or in combination with other agents also appear in this list.

Of the shortened list of ~50 drugs (combinations have been broken into individual drug entities) in Table 2, approximately half are devoid of structural alerts. These include both low (e.g., letrozole (**210**), risperidone (**212**), palonosetron (**228**), varenicline (**231**), etc.) and high daily dose drugs (e.g., emtricitabine (**188**), tenofovir (**189**), modafinil (**194**), topiramate (**195**), capecitabine (**222**), metoxolone (**224**), etc.). As such, there is no information in the literature concerning their propensity to form RMs, inhibit BSEP, etc., a phenomenon that appears to be consistent with their general tolerability (and lack of serious IADRs) in the clinic.

Foremost among drugs that feature structural alerts (Figure 15) include the previously discussed antidiabetic agents pioglitazine (**89**), rosiglitazone (**217**), and the tyrosine kinase inhibitors sunitinib (**50**) and erlotinib (**221**). Although rosiglitazone has not been associated with idiosyncratic hepatotoxicity, there are several reports linking rosiglitazone use with increased cardiovascular risks, and this has led to severe restriction in its use in the US. Like sunitinib and erlotinib, imatinib (**193**) has been associated with rare but severe cases of idiosyncratic hepatotoxicity.[346] Imatinib (**193**) possesses aniline/*para*-alkylaniline structural alerts; however, unlike erlotinib (Scheme 17), there is no evidence to suggest RM formation. The principal metabolic fate of imatinib in humans involves *N*-dealkylation of the *N*-methylpiperazine ring to a pharmacologically active metabolite.[347] Imatinib, however, is a potent BSEP inhibitor.[82]

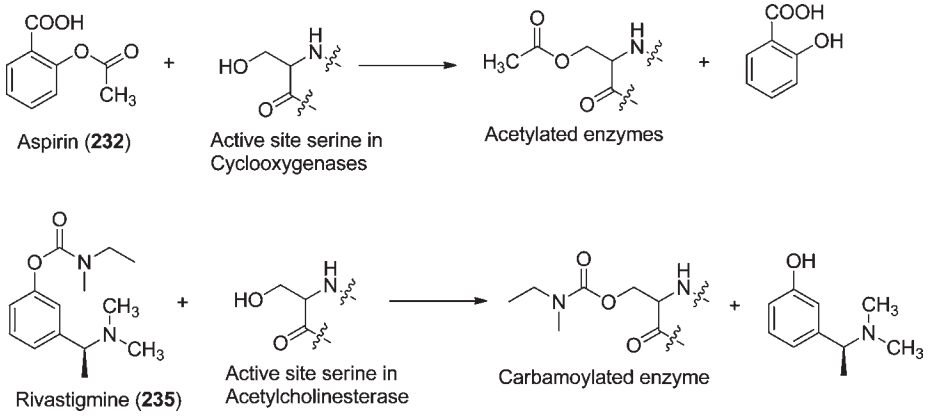
Antibacterial agents such as the β-lactam/β-lactamase inhibitor combination, piperacillin (**202**) and tazobactam (**203**), also make up the list. As described earlier, the pharmacologic basis of action of these compounds involves covalent interactions of the β-lactam motif with their respective biological targets. Moxifloxacin (**229**) is a fourth-generation fluoroquinolone antibiotic marketed to treat severe and life-threatening bacterial infections. Moxifloxacin does not contain the C-7 cyclopropylamine group found in trovafloxacin. In humans, the drug is primarily metabolized via glucuronidation and sulfation on the carboxylic acid and the cyclic secondary amine group, respectively.[348] The aniline and cyclopropylamine alerts on the fluoroquinolone motif in moxifloxacin are inert toward metabolism. However, it is interesting to note that moxifloxacin has been associated with rare cases of severe ADRs including QTc prolongation, irreversible peripheral neuropathy, acute liver failure or serious hepatotoxicity, and toxic epidermal necrolysis that appear to be immune-mediated.[349,350] The incidence of hepatic injury, however, is significantly greater than that seen with trovafloxacin.[351] The licensed uses for moxifloxacin are limited as moxifloxacin is considered to be a drug of last resort when all other antibiotics have failed. The biochemical basis for moxifloxacin-mediated IADRs remains to be characterized.

Linezolid (**207**) is a synthetic antibacterial agent from the oxazolidinone class of drugs and is used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to other antibiotics. In an acute setting (two weeks or less), linezolid is a relatively safe drug and can even be used in people with impaired hepatic and renal function. In humans, linezolid undergoes oxidative metabolism primarily on the morpholine ring, and the *bis*-aniline-type alert in linezolid is resistant to oxidative metabolism.[352]

Aspirin (**232**) has been used for over 100 years as an anti-inflammatory agent. The biochemical mechanism of the anti-inflammatory action of aspirin involves acetylation of an active site serine residue in the COX isozymes (Scheme 39).[353,354] The inactivated COX isoforms can no longer bind the fatty acid substrate arachidonic acid, and this leads to inhibition of pro-inflammatory prostaglandin production. While increased risk of gastrointestinal bleeding due to nonselective COX-1 and COX-2 inhibition is a well-known adverse event associated with aspirin use,[355] there are no reports of immune-mediated toxicity arising from indiscriminant acylation of proteins other than COX by aspirin.[356] Rivastigmine (**235**), which is used in the treatment of Alzheimer's disease, is a slow-binding, reversible inhibitor of the serine hydrolase, acetylcholinesterase. Co-crystalligraphic data of rivastigmine bound to the enzyme indicates the formation of the active site serine−carbamate adduct. The phenol leaving group is retained in the anionic site of the enzyme in proximity to the site of reaction.[357] There are no major safety liabilities associated with rivastigmine despite the potential for nonspecific protein carbamoylation.

Mesalazine (5-aminosalicylic acid, **208**) is widely used for the treatment of inflammatory bowel diseases and has largely

Scheme 39. Biochemical Mechanism of Pharmacologic Action for Aspirin (232) and Rivastigmine (235)



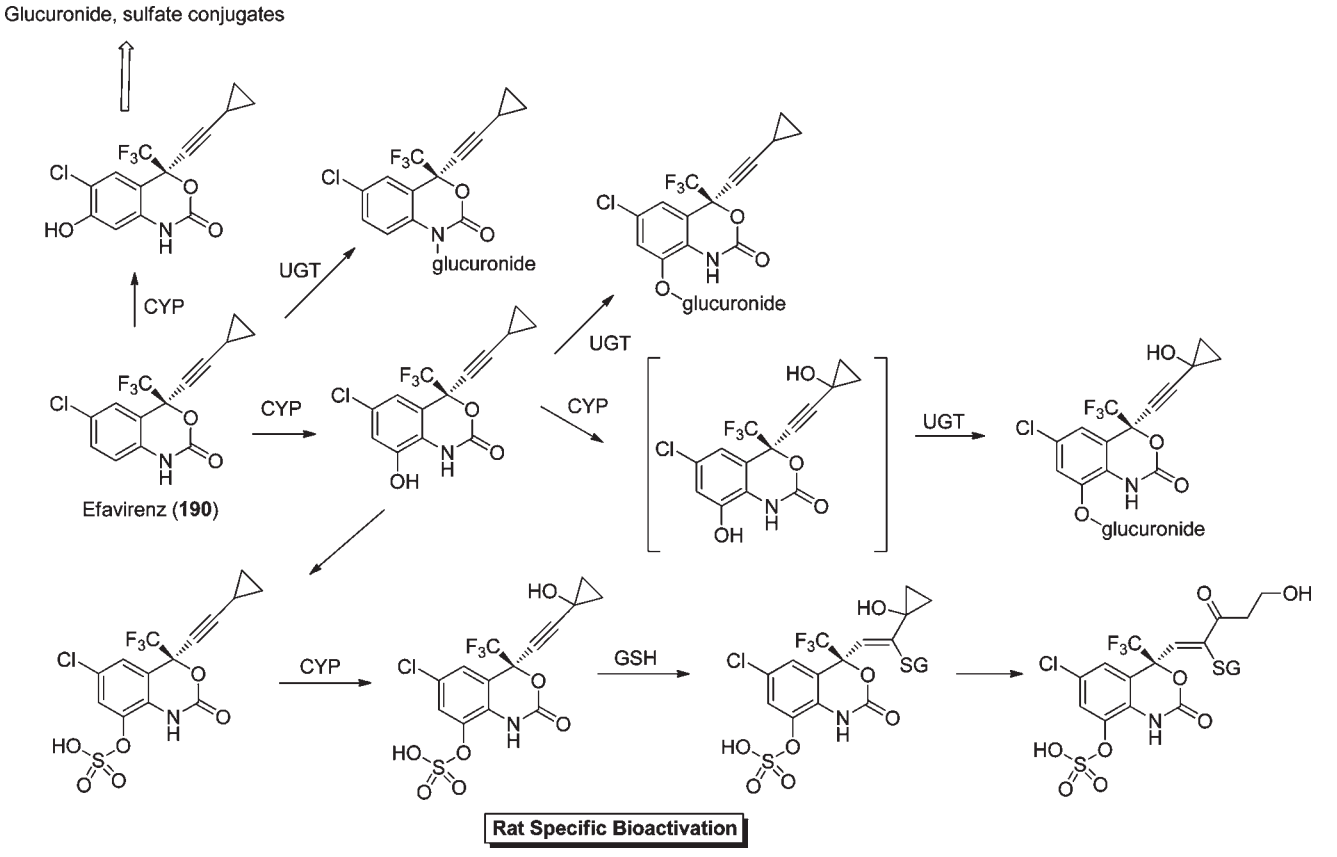
replaced sulfasalazine because of its improved safety. Mesalazine contains the *para*-aminophenol motif, which imparts antioxidant properties to the drug.[358] In the process of scavenging reactive oxygen species (e.g., hypochlorous acid generated in neutrophils), mesalazine is oxidized to an electrophilic quinone-imine, which is trapped by GSH.[359] In fact, there have been trace levels of oxidative products in feces of patients on mesalazine.[360] Mesalazine is primarily metabolized in humans via *N*-acetylation of the aniline nitrogen, which can be regarded as a detoxicating clearance mechanism.[361]

Efavirenz (**190**) is a potent and specific inhibitor of HIV-1 reverse transcriptase, approved for the treatment of HIV infection. Efavirenz is also used in combination with other antiretroviral agents such as the popular HIV medication Truvada, which consists of emtricitabine (**188**) and tenofovir (**189**) (Table 2, Supporting Information). At a high daily dose of 600 mg, the presence of the alkyne structural alert in efavirenz presents a potential safety risk in terms of RM formation. However, in humans, the alkyne functionality in efavirenz is latent toward oxidative metabolism; monohydroxylation is observed on the C-8 position on the aromatic ring and on the cyclopropyl methine (Scheme 40).[362] In contrast with the observations in humans, metabolism studies in rats have indicated the existence of a specific glutathione transferase capable of metabolizing downstream efavirenz products into a GSH conjugate.[362]

While *para*-alkylaniline and *ortho*-alkylaromatic ether alerts are present in ziprasidone (**192**) and atomoxetine (**216**) (Figure 16), respectively, human mass balance studies indicate that metabolism occurs on sites other than the structural alert motif.[363,364] Desvenlafaxine (**247**), the *O*-demethylated metabolite of venlafaxine (**93**), is a *para*-alkylphenol derivative with the potential to form a reactive quinone-methide species. However, as mentioned earlier, the principal route of desvenlafaxine metabolism in humans is via conjugation of the phenol functionality.[266] Pramipexole (**215**) (Figure 16) is a nonergot dopamine receptor agonist used in the treatment of Parkinson's disease. Urinary excretion of the parent is the major route (~ 90% of the daily dose of 4.5 mg) of pramipexole elimination in humans. Metabolite formation (including cleavage products of the 2-aminothiazole structural alert) has not been observed in circulation and/or urine (pramipexole package insert). The observation that the renal clearance of pramipexole is approximately three times greater than the glomerular filtration rate is consistent with the involvement of the organic cation transport system in its renal excretion.[365] Mycophenolic acid (**206**) (Figure 16) is widely used as an immunosuppressive agent after organ transplantation and in the treatment of autoimmune disease. In order to increase oral absorption, the drug is administered as an ester prodrug (mycophenolic acid mofetil). Its primary route of metabolism in humans involves glucuronidation of the phenol and the carboxylic acid metabolite that is obtained upon ester bond hydrolysis; oxidative metabolism on the *para*-alkylphenol and olefin alerts has not been observed in *in vivo* studies in human.[366] The antiretroviral agent darunavir (**246**) (Figure 16) contains the *para*-aminobenzenesulfonamide motif found in sulfamethoxazole. Darunavir is extensively metabolized in humans, and one of the circulating metabolites is the corresponding *ortho*-aminophenol derivative.[367] However, lack of detection of sulfydryl conjugates (e.g., mercapturic acid derivatives in urine) suggests that the further oxidation of the *ortho*-aminophenol to the *ortho*-quinone-imine species may not occur. Darunavir coadministered with ritonavir (**219**) has a warning for hepatotoxicity in the product label.

Budesonide (**199**) and triamcinolone (**248**) are steroidal agents used for the treatment of asthma and/or related allergies. They possess the quinonoid motif in the A ring, which can react with biological nucleophiles. However, similar to prednisolone and methylprednisolone, these compounds are primarily metabolized in humans via 6β-hydroxylation and oxidation(s) on the D ring.[368,369] Dutasteride (**211**) is a 5α-reductase inhibitor, which is currently used in the treatment of benign prostatic hyperplasia. Dutasteride features two alerts in its structure, an α,β-unsaturated lactam and anilide. Apart from 6β-hydroxylation as a primary metabolic fate in humans, there is information suggestive of metabolism on the structural alerts (e.g., formation of a *para*-hydroxyanilide metabolite and reduction of the olefin bond in the α,β-unsaturated lactam ring). Whether RMs can be generated from the oxidative metabolism process remains unknown, and the daily dose of dutasteride is very low (0.5 mg). Formoterol (**209**) is a long-acting β2-agonist used in combination with budesonide (**199**) for the management of asthma and/or chronic obstructive pulmonary disease. The drug contains *para*-alkoxyphenyl and *ortho*-hydroxyanilide motifs capable of forming reactive quinone-type species. However, *in vivo* metabolism studies in humans indicate that the principal elimination mechanism of formoterol involves conjugation to inactive glucuronides and a sulfate derivative.[370] The phenol glucuronide of formoterol is the main metabolite detected in urine. While *O*-demethylation and *N*-deformylation is observed, overall plasma exposure to these metabolites is low because formoterol is a low dose drug administered via inhalation.

Scheme 40. Species Differences in the Oxidative Bioactivation of Efavirenz (190) in Humans and Rats



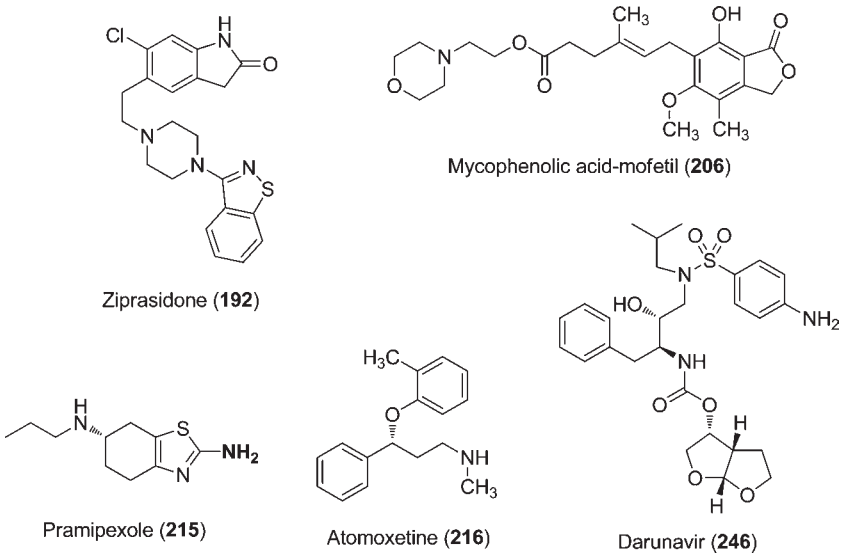


Figure 16. Examples of drugs which contain structural alerts but are not prone to RM formation.

9.0. LESSONS LEARNED FROM THE STRUCTURAL ALERT/RM ANALYSES FOR DRUGS ASSOCIATED WITH IADRS AND THE TOP 200 DRUGS OF 2009

Collectively, our analysis on 68 drugs associated with idiosyncratic toxicity revealed that a significant proportion (~78−86%) contained structural alerts, and evidence indicating RM formation as a causative factor for toxicity has been presented for 62−69% of these molecules. Of all the chemical groupings identified as possible structural alerts, the aniline/anilide motif is best known to be associated with mutagenicity, direct toxicity, methemoglobinemia,

dx.doi.org/10.1021/tx200168d |*Chem. Res. Toxicol.* 2011, 24, 1345–1410

and immunogenic allergenic toxicity. The motif is widely used in kinase inhibitor design as evident with the tyrosine kinase inhibitors (e.g., lapatinib, sunitinib, and erlotinib) that are associated with significant incidences of hepatotoxicity, and RM issues continue to emerge in kinase programs, which rely on the aniline group.[371] While at first glance, it appears as though the presence of a structural alert in a compound should accurately predict toxicity potential, it is noteworthy to point out that the concept of structural alerts as defined in modern medicinal chemistry originated from bioactivation studies with many of the drugs presented in Tables 1 and 2. Thus, it is not surprising that a strong relationship exists between RM formation, protein covalent binding, and idiosyncratic toxicity with these agents. A compelling argument for chemotype-specific toxicity is also evident from the structure−activity analyses (e.g., alpidem (**23**) versus zolpidem (**101**), trovafloxacin (**31**) versus levofloxacin (**109**), clozapine (**39**) versus quetiapine (**87**), nefazodone (**48**) versus buspirone, etc.), wherein absence of RM liability appears to be consistent with the improved safety profile of successor drugs. Although anecdotal for the most part, the structure−toxicity relationships support the notion that avoiding structural alerts in drug design would lead to therapeutic agents devoid of idiosyncratic toxicities. In fact, knowledge-based systems such as Derek for Windows, that are used to predict the toxicity of a chemical from its structure, have evolved from such findings.[372] Predictions from Derek, however, can be misleading at times. For instance, 2-aminopyridine and 2-aminopyrimidine functionalities are not predicted to be structural alerts from Derek and are commonly used in medicinal chemistry as aniline replacements. Certainly, the 2-aminopyrimidine motif found in buspirone is devoid of RM issues, unlike the aniline derivative and RM positive drug nefazodone. However, an exception to this rule is the antibacterial agent trimethoprim (**58**) (Scheme 21), where the formation of the imine-methide species requires a formal two-electron oxidation of the 2-aminopyrimidine nucleus. As novel (and proprietary) functional groups are continuously sought in drug design, it is possible that unanticipated bioactivation pathways leading to RMs will emerge. Certainly, this has been the topic of many excellent reports in this journal and medicinal chemistry journals.[373−376]

Our analysis also clearly indicates that approximately half of the top 200 drugs for 2009 (prescription and sales) contain one or more alerts in their chemical architecture. With relatively few exceptions (e.g., lamotrigine (**45**), sulfamethoxazole (**54**)/trimethoprim (**58**), acetaminophen (**70**), furosemide (**96**), and amitriptyline (**158**)), the vast majority of the structural alert-containing drugs are rarely associated with significant IADR incidences, despite years of use. These observations indicate that the mere presence of a structural alert in a molecule will not necessarily trigger RM formation. Thus, from a drug discovery perspective, it is imperative to demonstrate experimentally whether structural alerts, if present in a candidate of interest, actually are prone to RM formation. Metabolism can occur at a site other than the structural alert and lead to nonreactive products. For example, both sudoxicam (**11**) and meloxicam (**151**) contain the 2-aminothiazole structural alert, but only sudoxicam forms the reactive acylthiourea (Scheme 6). Likewise, entacapone contains the nitrocatechol motif present in tolcapone (**13**) but does not generate electrophilic quinone-imine species detected with tolcapone (Scheme 7). Additional diligence regarding the possibility of nonmetabolic routes of clearance (e.g., biliary and/or renal excretion of parent drug), which can supersede metabolism (including RM formation) should also be taken into consideration. For instance, drugs that feature structural alerts such as rosuvastatin (**90**), tiotropium (**136**), olmesartan (**139**), ranitidine (**187**), pramipexole (**215**), etc. are subject to extensive renal excretion in humans (often mediated by transport proteins) and are generally resistant to metabolism/bioactivation.

As is the case with any analysis, the outliers (both false positives and false negatives) were of great interest. These drugs challenge the notion of structural alert and/or *in vitro*/*in vivo* RM analysis as standalone predictors of toxicity. While the weight of evidence suggests that RMs are responsible for many IADRs, it is important to note that virtually any functional group, including a phenyl ring, can generate a RM and/or covalently bind to protein in *in vitro* systems (e.g., HLM) when measured with sensitive state-of-the-art bioanalytical methodology. Certainly, this appears to be the case for several marketed drugs in the prescription (e.g., atorvastatin (**71**), esomeprazole (**75**), clopidogrel (**76**), montelukast (**78**), simvastatin (**81**), ethinyl estradiol (**122**), losartan (**128**), paroxetine (**154**), raloxifene (**183**), etc.) and sales (e.g., aripiprazole (**88**), pioglitazone (**89**), duloxetine (**92**), olanzapine (**94**), etc.) categories, all of which contain a structural alert(s). As noted earlier, in the case of clopidogrel, RM formation by CYP is essential for its antiplatelet effects. It is very likely that the false positive rate for RM formation will increase if all of the top 200 structural alert-containing drugs are tested in these assays. For example, clonazepam (**107**), donepezil (**117**), dutasteride (**211**), darunavir (**246**), etc. contain structural alerts; however, their propensity to form RMs has not been examined in *in vitro* systems such as HLM. A clear message from these observations is that RM detection assays (exogenous trapping with nucleophiles) are not intended to predict toxicity but rather to detect the formation of RMs, some of which may carry a toxic liability. Reducing exposure to such potential toxins is viewed as one approach to minimize risk during drug development.

Overall, this discrepancy raises a fundamental question: why are some RM-positive drugs safe, while others are not? A limitation of the *in vitro* RM screens is that they are typically conducted in HLM (in the presence and absence of NADPH cofactor), which only examines CYP-catalyzed RM formation. In some instances, RM formation may be observed in microsomes in a CYP-dependent fashion, but *in vivo*, the compound may undergo a distinctly different and perhaps more facile metabolic fate that circumvents CYP catalysis. For instance, losartan (**128**) forms GSH adducts derived from the CYP-mediated bioactivation of the alkylimidazole alert, which leads to microsomal covalent binding. However, *in vivo*, losartan is cleared through oxidation of the primary alcohol moiety, tetrazole-*N*-glucuronidation, and biliary excretion in parent form. The widely prescribed drugs paroxetine (**154**) and raloxifene (**183**) serve as additional examples of this phenomenon. Both drugs form GSH conjugates and covalently bind to HLM in a CYP-dependent fashion; however, *in vivo*, the quinone precursors are principally metabolized via competing glucuronidation and/or *O*-methylation pathways. It is tempting to speculate that in the modern drug discovery paradigm, paroxetine and raloxifene would unlikely be considered as candidates for clinical development because of the high degree of microsomal covalent binding and GSH adduct formation. To minimize false positives, our analysis suggests that RM assessments in HLM should be followed by more detailed studies in fully integrated *in vitro* biological matrices such as hepatocytes and S-9 fractions from both human and animal species. Establishing a clear understanding of the *in vivo* clearance mechanisms in animals and how that relates to RM formation

in vitro matrices would lead to data-driven decision making with regards to compound selection. An additional consideration of much importance is the clinical indication. The level of risk that would be deemed acceptable for drug candidates intended to treat a major unmet medical need and/or a life-threatening disease is likely to be significantly higher relative to the treatment of chronic nondebilitating conditions where alternate treatment options are already available. An excellent illustration of this phenomenon is evident with the tyrosine kinase inhibitor sunitinib, which is associated with a BBW for hepatotoxicity but is widely used in cancer treatment.

It is also striking that high dose drugs (>100 mg) tend to be the ones, which most frequently cause IADRs, while low dose drugs (<50 mg) rarely are problematic in this regard (whether these agents are prone to RM formation). Examples of this phenomenon are readily apparent with the amineptine/tianeptine, clozapine/olanzapine, troglitazone/pioglitazone, nefazodone/aripiprazole, and amitriptyline/cyclobenzaprine pairs discussed earlier. As such, the vast majority of structural-alert-positive drugs in the top 200 list (prescription/sales) are also low dose drugs. The improved safety of low dose drugs (olanzapine, pioglitazone, and aripiprazole) could arise from a marked reduction in the total body burden to RM exposure via efficient scavenging by GSH (and other competing metabolic pathways), such that the reactive species are unlikely to exceed the safety threshold needed for toxicity.[92,255,256] This may well be the case for clopidogrel whose principal clearance mechanism proceeds via ester bond hydrolysis; consequently, the amount of RM produced (~ 0.2% of the administered dose) must not exceed the threshold for toxicity. Given this general trend, optimization of lead compounds in drug discovery programs typically focus on improving pharmacokinetics and intrinsic potency as a means of decreasing the projected clinically efficacious plasma concentrations (and hence the dose) and the associated body burden of parent drug and its metabolites as a strategy for mitigating IADR risks. However, it is clear that there will be classes of drugs (e.g., antibacterials, antiretrovirals, etc.), where this will be difficult to achieve (see Supporting Information Table 2 for illustrations). A few exceptions to the "low dose" rule were also noted in our analysis (bromfenac (**3**), pipamazine (**21**), ambrisentan (**32**), and leflunomide (**66**) with daily doses <50 mg). While the reason for this discrepancy is unclear, it is interesting to note that, with the exception of bromfenac, there is no evidence for RM formation with the other drugs.

Recent advances in risk assessment methodologies, such as the estimate of total daily body burden of covalent binding in hepatocytes or by zone classification taking the clinical dose into consideration, are positive steps toward quantitative prediction of IADR risks with drug candidates.[92,255,256] The zone classification system for IADR risk prediction suggests that drugs could be placed into one of three zones (safe, problematic or unacceptable) based on the fraction of metabolic clearance resulting in HLM covalent binding and daily dose information.[92] For instance, olanzapine exhibits higher covalent binding than zomepirac in human hepatocytes (covalent binding for olanzapine and zomepirac is estimated to be 38.5 ± 0.9 pmol/mg protein and 7.2 ± 0.4, respectively), but olanzapine is placed in the safe zone, while zomepirac is placed in the problematic zone when the daily doses of olanzapine (10 mg) and zomepirac (600 mg) are taken into consideration.

Experiments that define the relationship between drug bioactivation (e.g., the *in vivo* formation of GSH conjugates) and toxicity in humans are extremely rare.[92,377,378] Although GSH adducts and/or downstream mercapturic acid metabolites that are measured *in vivo* represent short-term exposure to RMs, protein adducts reflect the internal exposure of cells to RMs *in vivo*, which is more relevant for risk assessment purposes. Whether covalent binding measures *in vivo* is likely to be more informative of the *in vivo* safety risk than covalent binding studies *in vitro* remains to be established. This is because of a lack of data on absolute levels of *in vivo* covalent binding that could lead to a toxic outcome versus levels of binding that are safe. As such, next to dosimetry and quantitative considerations of RM exposure, identifying toxicologically relevant protein targets of RMs is an important challenge to a better understanding of the links between covalent binding to critical proteins and organ toxicity.[379] Which protein targets are critical for toxicity is, of course, a key question and one to which we do not currently have answers. However, methodology to identify the proteins that are subject to covalent modification by electrophilic drug metabolites is now available, thanks to advances in proteomics mass spectrometry, and databases of the type established by Professor Bob Hanzlik and colleagues at the University of Kansas ultimately will reveal which protein targets are critical to cell survival following alkylation.[380] Perhaps the biggest gap in the field is the lack of knowledge about the nature of the antigenic determinants that trigger an immunological response to drug−protein covalent adducts. Without this information, it will not be possible to predict the potential for a given compound that undergoes metabolic activation to cause idiosyncratic toxicity in humans through haptenization of key proteins.

Within this context, it is important to point out that the most common chemical outcome of bioactivation is bioinactivation, usually via the conjugation of RMs with GSH or another detoxication system (e.g., hydrolysis of electrophilic epoxides by epoxide hydrolases). In addition, mammals possess efficient cell defense systems for adapting to chemical stress posed by RMs. For example, upon exposure to acetaminophen, an array of transcription factors and signaling proteins have been implicated in sensing and potentially adapting to chemical stress with associated endogenous perturbation. Although some proteins may be more important than others in determining the physiological response to stress, and there will be a degree of chemical specificity in driving this response, it is clear that no single pathway acts as the ultimate sole determinant of adaptation. Nevertheless, the induction of several key transcription factor pathways, such as nuclear receptor factor 2 (NRF2), is clearly an important mechanism for adaptation to chemical toxicity, and through reactivity of key cysteine residues in the inhibitor of NRF2−Kelch-like ECH-associated protein 1, this pathway can sense chemical danger and orchestrate cell defense. Ultimately, it would be valuable to define clinical (and preclinical) mechanism-based and translational biomarkers that will assist in understanding the basic biology that determines how impending stress is sensed.[381]

While necessary diligence is observed toward the discovery of a RM-negative candidate with the optimal balance of pharmacology and pharmacokinetics, it is noteworthy to point out that a negative finding in the RM and/or covalent binding assays is not a guarantee of safety. For example, the felbamate (**64**) bioactivation process in humans (Scheme 24), which leads to the formation of the electrophilic 2-phenylpropenal has never been replicated *in vitro* (e.g., HLM, hepatocytes, etc.). At therapeutically relevant concentrations of radiolabeled felbamate, no GSH adducts and/or covalent binding has been discerned in HLM or

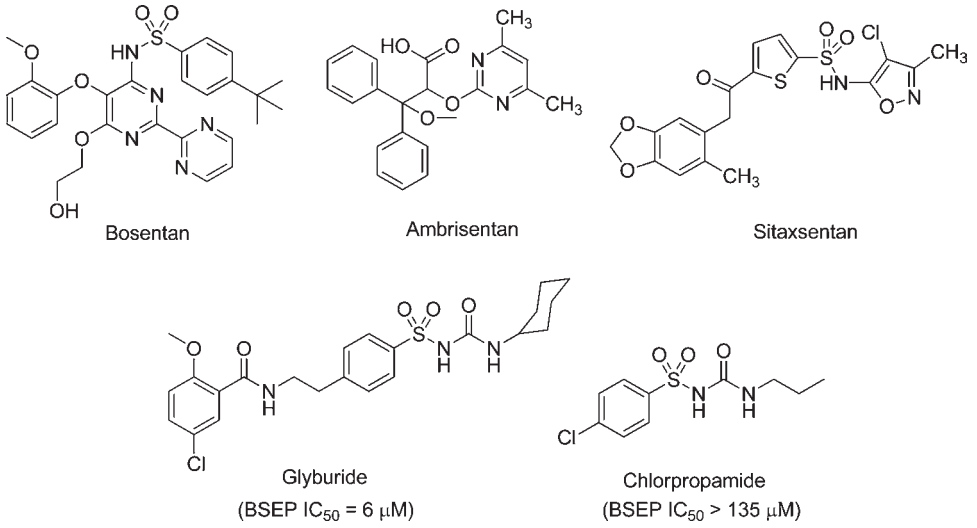


Figure 17. Structure−activity relationship for BSEP inhibition.

human hepatocytes.[382] While the reason for this discrepancy remains unclear, in a drug discovery paradigm relying solely on RM trapping and liver microsomal covalent binding as means of predicting drug toxicity, felbamate would have passed the hurdle. Likewise, ximelagatran (**22**) does not contain any structural alerts and is not prone to RM formation, yet the drug was associated with several cases of hepatotoxicity, which led to its withdrawal. The findings with ximelagatran are a strong testimony to the fact that a drug candidate devoid of RM formation and/or covalent binding to proteins is not a guarantee of its safety. Overall, the risk of RM formation is only part of a multifaceted, integrated risk-benefit assessment of a drug candidate and should not serve as a stand-alone criterion to suspend the development of a clinical candidate.

Of much interest in our analysis are the observations on the multifactorial aspects of idiosyncratic hepatotoxicity. For example, in addition to RM generation, drugs such as amineptine (**1**), tolcapone (**13**), alpidem (**23**), troglitazone (**30**), ketoconazole (**43**), and nefazodone (**48**) demonstrate mitochondrial toxicity. An interesting structure−activity relationship also emerges with tolcapone, alpidem, and nefazodone, upon the basis of the findings that structurally-related drugs with improved safety (entacapone, zolpidem, and buspirone) do not form RMs and do not impair mitochondrial function. High-throughput *in vitro* systems (isolated organelles, HepG2 cells, etc.) are available to screen for mitochondrial toxicity in drug discovery,[16,17] although some questions can be raised on the ability to prospectively predict idiosyncratic toxicity using *in vitro* data. For example, buprenorphine (**182**) impairs mitochondrial respiration and ATP formation[320] but does not cause hepatotoxicity at its recommended doses of 2−8 mg. To date, assessment of drug-induced mitochondrial toxicity has been mostly retrospective and mechanistic in nature, focusing on problematic drugs. The analysis has been essential to establish limits of predictability, such as determining how severe a mitochondrial impairment has to be before it leads to a toxicological outcome.

Equally provocative in our analysis was the emergence of BSEP inhibition as a contributor to cholestatic injury observed with several drugs including troglitazone (**30**), ketoconazole (**43**), lapatinib (**44**), nefazodone (**48**), pazopanib (**50**), bosantan (**59**), glyburide (**166**), imatinib (**193**), ritonavir (**219**), etc. Despite a lower daily dose (2.5−20 mg), glyburide is associated with much greater incidence and severity of hepatic injury than any of the sulfonyl ureas. Compared with the older hydrophilic sulfonyl ureas such as chlorpropamide (Figure 17), which are mainly excreted renally, glyburide is more lipophilic and undergoes significant biliary excretion, possibly mediated through active hepatobiliary transport. While glyburide is devoid of structural alerts, the drug is a potent inhibitor of BSEP (IC_{50} ~6 μM) compared with related sulfonyl ureas such as chlorpropamide (IC_{50} ~135 μM).[268] All evidence thus points to the greater inhibitory potency of glyburide against hepatobiliary transporters as a key determinant of its cholestatic potential.

Endothelin receptor antagonists bosentan (**59**), ambrisentan (**32**), and sitaxsentan (Figure 17), which are used in the treatment of pulmonary hypertension, initially had BBWs for hepatotoxicity risks and require discontinuation of treatment if elevation of liver enzymes is accompanied by clinical signs of hepatotoxicity. It has been argued that hepatic injury is a class effect of these drugs, although there is no formal evidence suggesting that pharmacologic mechanism plays a role. The removal for the BBW for ambrisentan casts further doubt on this being a class effect. While ambrisentan does not inhibit BSEP, the first-in-class agent bosentan is a potent BSEP inhibitor in humans and rats, findings that are consistent with elevated plasma bile salts in both species. More recently, inhibition of hepatobiliary transport by sitaxentan also has been demonstrated.[110] However, in the case of sitaxsentan, a small number of cases of severe hepatotoxicity leading to fatalities have been reported, which has resulted in the recent withdrawal of this drug from European markets.[383] These cases appear to be distinct from the typical cholestasis associated with BSEP inhibition, in that liver enzymes remained elevated even after drug withdrawal, and liver biopsies indicated hepatocellular damage and necrosis. While the underlying mechanism leading to severe toxicity remains unknown, it is important to note that there may be additional risk factors at play here beyond BSEP inhibition. For example, the principle clearance mechanism of sitaxentan in humans involves metabolism of its 1,3-benzdioxole structural alert (Figure 17) to yield a catechol metabolite capable of further oxidation to a reactive quinone species. The clinically efficacious daily dose of sitaxsentan is ~100 mg.

Screens for BSEP inhibition are available in a drug discovery setting and can prove useful toward establishing structure−activity

relationships as was shown with toxic nefazodone and nontoxic buspirone.[160,384] However, as in the case of RM positives, interpretation of BSEP inhibition data requires additional scrutiny. For example, *in vitro* BSEP inhibition seen with troglitazone has also been noted with rosiglitazone and pioglitazone, which are not hepatotoxic. Likewise, simvastatin (**81**) and ethinyl estradiol (**122**) exhibit potent BSEP inhibition but are not associated with cholestatic injury in humans. It is likely that the therapeutic concentrations of these drugs in the liver are significantly below the IC_{50} values for inhibition of BSEP activity in the liver. This brings up the question of whether a low dose drug (leading to low hepatic and systemic concentrations) would mitigate idiosyncratic hepatotoxicity risks due to BSEP inhibition alone (in a manner similar to that of RM formation). From a drug discovery viewpoint, determination of the BSEP IC_{50}/projected portal inlet drug concentration (surrogate of liver concentrations) ratio could provide a therapeutic index to derisk cholestatic potential due to BSEP inhibition. However, such derisking strategies will need validation prior to introduction in a drug discovery setting for risk mitigation of cholestatic injury due to BSEP inhibition. It should also be noted that oxidative stress,[385] certain disease states, pregnancy, and genetic mutations[386] may confer additive risk factors beyond simple potency estimates of inhibition. Recommendations from the International Transporter Consortium with regards to decision trees that are intended to help guide clinical studies on drug transporter interactions have been recently reviewed[387] and could be potentially expanded to include derisking strategies for BSEP inhibitors.

Despite a steady increase in pharmaceutical R & D spending, the number of drug approvals has declined in recent years. Toxicity continues to account for over 30% of attrition in drug development and remains one of the major causes for drug recall and/or restrictions in use. R & D costs increase exponentially in the late stage drug development, and identification of the toxicological potential of new chemical entities at an early stage should allow R & D resources to be invested in the drug candidates most likely to succeed. Greene and Naven[388] have recently reviewed *in vitro* safety screening assays (e.g., *Salmonella* Ames, *in vitro* micronucleus, hERG patch clamp assays, CYP inhibition, etc.) used to identify chemotype risks. Off-target pharmacology screens also serve as a filter, and compounds that exhibit promiscuity can be removed from further consideration. In many such situations, there is usually a strong relationship between well-defined toxicity mechanisms and discrete *in vivo* end points that can be used as decision making tools for compound progression. For this purpose, acute, repeat dose *in vivo* toleration studies are being conducted as early as possible to minimize and/or exclude *overt* toxicity risks due to the pharmacologic mechanism and/or chemotype. Animals are poor predictors of IADRs in humans, and because RMs have been implicated in the etiology of many IADRs, RM screens also have been implemented to assist in compound design. While this is a useful and pragmatic starting point toward eliminating potential IADR risks, our analysis suggests that in order to truly mitigate *covert* toxicity risks (particularly idiosyncratic hepatotoxicity) there is a need for a more integrated *in vitro* screening paradigm that involves examination of bioactivation potential as well as cellular effects mediated by the parent compound (and its metabolites). Consistent with this viewpoint, many companies have advocated the use of cell-based (e.g., ATP depletion in transformed human liver epithelial cells) cytotoxicity measures, BSEP inhibition, mitochondrial toxicity, and RM assessments in a drug discovery paradigm.[389−392] With the plethora of new *in vitro* safety screens available, it is likely that drug discovery teams will tend to overinterpret the output from these assays, the primary purpose of which is merely to identify chemotype hazard risks and not predict idiosyncratic toxicity. Consequently, how such a collective output would be used in the holistic assessment of IADR risks will ultimately rest with the individual companies. For instance, investigators at Astra Zeneca[389] have proposed the concept of a hepatic liability score, derived from the summation of toxicity scores for a test compound in the various *in vitro* assays. Plotting the liability score against an estimated RM body burden score, which is determined from the zone classification process, then assesses IADR risks. While such an approach clearly needs validation, it is noteworthy to point out that troglitazone, in contrast to rosiglitazone and pioglitazone, would be pinpointed as a clear hazard in this model, despite having similar potency values in some assays (e.g., BSEP inhibition). Troglitazone is positive in BSEP inhibition and mitochondrial dysfunction, and by contrast, cytotoxicity assessments gives it a high hepatic liability score. Moreover, it lies in the problematic zone in the zone classification method, on the basis of high covalent binding potential and high daily dose. Until we gain a better understanding of the biochemical mechanisms that underline IADRs in the clinic, such *in vitro* safety predictors will remain a critical issue for further investigation and debate.

■ ASSOCIATED CONTENT

Supporting Information. Structures, indication, physiochemical attributes, structural alerts, RM data, and the maximum recommended daily doses of the top 180 drugs in the US market by prescription and by sales in 2009. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

IADRs, idiosyncratic adverse drug reactions; HLA, human leukocyte antigen; RM, reactive metabolite; CYP, cytochrome P450; GSH, reduced glutathione; BBW, black box warning; MW, molecular weight; TPSA, topological polar surface area; NSAID, nonsteroidal anti-inflammatory drug; AUC, plasma area under the curve; HLM, human liver microsomes; COX, cyclooxygenase; COMT, catechol-*O*-methyl transferase; UGT, uridine diphospho glucuronosyltransferase; MPO, myeloperoxidase; NAT, *N*-acetyltransferase; FMO, flavin monooxygenase; DDI, drug−drug interaction; PMN, polymorphonuclear leukocytes.

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