Special Section on Perspectives in Drug Metabolism and Disposition

Cytochrome P450 and Other Drug Metabolizing Enzymes as Therapeutic Targets

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Abbreviations: GPCR, G-protein coupled receptor; P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; SULT, sulfortransferase; PAPS, 3-phosphoadenosine-5-phosphosulfate; GST, glutathione S-transferase; RAMBA, retinoic acid metabolism blocking agent; AhR arylhydrocarbon receptor; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; mEH, microsomal epoxide hydrolase; sEH, soluble epoxide hydrolase; MAO, monoamine oxidase; AKR, aldo-keto reductase; AO, aldehyde oxidase; XO, xanthine oxidase; TMDD, target-mediated drug disposition.
Abstract

Cytochrome P450 and other families of drug metabolizing enzymes are commonly thought of and studied for their ability to metabolize xenobiotics and other foreign entities as they are eliminated from the body. Equally as important, however, is the homeostatic role that many of these enzymes play in maintaining the proper levels of endogenous signaling molecules such as lipids, steroids, and eicosanoids, as well as their ability to modulate protein-protein interactions involved in downstream signaling cascades. Throughout the years, many of these endogenous ligands or protein partners of drug metabolizing enzymes have been associated with a wide range of disease states from cancer to various cardiovascular, neurological or inflammatory diseases, prompting an interest in whether or not modulation of drug metabolizing enzyme activity could have a subsequent pharmacological impact or lessening of disease severity. Beyond direct regulation of endogenous pathways, drug metabolizing enzymes have also been proactively targeted for their ability to activate pro-drugs with subsequent pharmacological activity or enhance the efficacy of a co-administered drug by inhibiting the metabolism of that drug through a rationally designed drug-drug interaction (i.e., ritonavir and HIV antiretroviral therapy). The focus of this minireview will be to highlight research aimed at characterizing cytochrome P450 and other drug metabolizing enzymes as therapeutic targets. Examples of successfully marketed drugs as well as early research efforts will be discussed. Finally, emerging areas of research utilizing typical drug metabolizing enzymes to impact clinical outcomes will be discussed.
Significance Statement

While generally thought of for their drug metabolizing capabilities, enzymes such as the cytochromes P450, glutathione S-transferases, soluble epoxide hydrolases and others play a significant role in regulating key endogenous pathways, making them potential drug targets. This minireview will cover various efforts over the years to modulate drug metabolizing enzyme activity towards pharmacological outcomes.
Introduction

The drug discovery process generally starts with the identification and validation of a druggable target with the ability to affect a given disease outcome. Common classes of drug targets include G-protein coupled receptors (GPCRs), ion channels, kinases and nuclear receptors (Overington et al., 2006; Santos et al., 2017). In addition to these classes, enzymes such as angiotensin-converting enzyme, 3-hydroxy-3-glutaryl coenzyme A, the aspartic protease of HIV-1 and other enzymes have been noted to account for at least a third of the drug targets currently under evaluation (Holdgate et al., 2018). While enzymes specific to xenobiotic metabolism (cytochrome P450, UDP-glucuronosyltrasnferase, glutathione S-transferase, etc) are more commonly thought of when optimizing the ADME properties of a drug development candidate, their role in regulating endogenous processes has led researchers to evaluate these enzymes as potential drug targets (Guengerich, 2017).

The cytochrome P450 (P450) family of heme-containing enzymes are the primary enzymes involved in catalyzing the metabolic elimination of the majority of therapeutic drugs (Guengerich, 2005). Over 60 individual P450 isoforms have been identified, though only ten or so (CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5) are considered to be relevant in the clearance of xenobiotics (Wrighton and Stevens, 1992). P450 enzymes are ubiquitous to the body, with the liver and intestine being key sites of drug metabolizing activity (Paine et al., 2006; Hines, 2007; Thelen and Dressman, 2009; McDonnell and Dang, 2013; Sadler et al., 2016). Additional sites of expression include the kidney, brain, lungs and lining of the nasal mucosa (Ding and Kaminsky, 2003). Recent focus areas for scientists studying P450 enzymology from an ADME standpoint have included the contributions to drug metabolism by less studied P450s such as CYP1A1, CYP2J2 or those comprising the CYP4F family, as well as the role that P450s and other drug metabolizing enzymes can play in affecting the concentration of a drug at the therapeutic site of action (ie., brain, tumor, skin) (Foti et al., 2015; Klomp et al., 2020; Sisignano et al., 2020). As the P450 superfamily of heme-containing enzymes is arguably the most well-studied of the known drug metabolizing enzymes, it is not surprising that a
considerable effort has been placed on understanding the role of P450s in regulating endogenous pathways. Researchers have long postulated roles for P450s in affecting the severity of cancer progression, atherosclerosis, diabetes and various neurological diseases, prompting additional research into the potential of P450 modulation as a potential therapeutic strategy (Ahmad and Mukhtar, 2004; Nebert and Dalton, 2006; Fan et al., 2015; Haduch and Daniel, 2018; Imig, 2018; Navarro-Mabaraku et al., 2018).

Beyond P450-catalyzed metabolic pathways, the UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) enzymes are well characterized enzyme families which contribute to much of the “Phase 2” or conjugative metabolism of xenobiotics (Burchell and Coughtrie, 1989; King et al., 2000; Gamage et al., 2006; Jancova et al., 2010). UGTs increase the hydrophilicity of their substrates (aglycones) by catalyzing the addition of glucuronic acid from UDPGA (uridine diphosphoglucuronic acid) to the aglycone, thus facilitating the renal elimination of the resulting metabolites (Radominska-Pandya et al., 1999). Xenobiotics or their oxidative metabolites that contain a nucleophilic functional group such as a carboxylic acid, phenol, aliphatic alcohol or other groups can undergo UGT-catalyzed metabolism and similar to P450s, their role in the regulation of endogenous molecules including fatty acids, estrogens and bilirubin has been studied in depth (Bock, 2015). Toward the possibility of utilizing UGTs as a therapeutic target, recent research has begun to focus on the role that UGTs play in the regulation of endogenous molecules involved in tumorigenesis and progression (Allain et al., 2020a). In a similar vein, SULTs increase the hydrophilicity of their target molecules by catalyzing the transfer of a sulfonyl moiety from PAPS (3-phosphoadenosine-5-phosphosulfate) to a nucleophilic functional group, many of which are the same as those noted above for UGTs (Foti et al., 2012). SULTs are divided into membrane-bound and cytosolic groupings, with the metabolism of xenobiotics generally attributed to the latter (Gamage et al., 2006). From a homeostatic regulation standpoint, both families are involved in the metabolism of endogenous molecules including neurotransmitters, fatty acids, steroids, lipids and glycosaminoglycans (Park-Chung et al., 1999; Mueller et al., 2015; Coughtrie, 2016).
expression of various SULTs and their resulting activity has recently been implicated in the severity and prognosis of multiple cancer types.

With regard to studying the role of drug metabolizing enzymes in the regulation of disease mechanisms and the subsequent potential to utilize them in therapeutic regimens, the glutathione S-transferase (GST) enzymes have also received a significant amount of attention to date. GSTs (both membrane-bound and cytosolic) catalyze the addition of glutathione (GSH) to an electrophilic substrate, resulting in a key detoxification and elimination mechanism for a multitude of substrates (Foti et al., 2012). Well-studied GST substrates include aflatoxin B1, cisplatin, acrolein and 4-hydroxynonenal (Allocati et al., 2018). Endogenously, GSTs are involved in the metabolism of prostaglandins, estrogens and leukotrienes and play a role in the regulation of the MAP kinase pathway through the modulation of protein-protein interactions, suggesting the potential to modify GST activity and impart subsequent pharmacological outcomes (Kyriakis et al., 1994; Adler et al., 1999; Tew and Ronai, 1999; Wang et al., 2001; Cuadrado et al., 2003; Elsby et al., 2003; Lushchak, 2012).

The following sections of this minireview will highlight research around the potential for drug metabolizing enzymes to be used as drug targets against various diseases. In addition to the enzyme families mentioned above, brief overviews of research efforts focused on epoxide hydrolases, monoamine oxidases, aldo-keto reductases, and xanthine oxidase as potential drug targets will also be included. The review will cover drugs that have been approved by regulatory agencies as well as many of those currently in pre-clinical or clinical development. Finally, recent areas of research utilizing typical drug metabolizing enzymes to impact clinical outcomes (ie., prodrug activation or clearance reduction strategies via co-administered enzyme inhibitors) will be discussed.

Brief Historical Perspective and Key Recent Advances

Cytochrome P450
A recent review on current drug targets suggests that 3.45% of compounds in the ChEMBL database (https://www.ebi.ac.uk/chembl/) and 0.84% of approved drugs were designed to therapeutically target cytochrome P450 enzymes (Santos et al., 2017). Indeed, numerous efforts have been described around targeting P450s with cancer therapies owing to their role in tumor formation (tumorigenesis) and tumor growth (Bruno and Njar, 2007). CYP17A1 (17α-hydroxylase; C17,20-lyase) and CYP19A1 (aromatase) have long been studied given their role in the regulation of androgens and estrogens (Figure 1). CYP17A1 converts endogenous pregnenolone and progesterone to 17α-hydroxypregnenalone and 17-hydroxyprogesterone, respectively, and is involved in the subsequent conversion of 17-hydroxyprogesterone to dehydroepiandrosterone (DHEA). CYP19A1 catalyzes the oxidation of androstenedione to estrone and of testosterone to 17β-estradiol (Guengerich, 2005). Clinically, abiraterone and ketoconazole have been utilized as inhibitors of CYP17A1 (Figure 2, Table 1) (Trump et al., 1989; Attard et al., 2008; Garrido et al., 2014; Gomez et al., 2015; Malikova et al., 2017). Abiraterone has shown clinical efficacy in castration-resistant prostate cancer, however it was not effective in treating estrogen receptor positive (ER+) metastatic breast cancer (O'Shaughnessy et al., 2016). Drugs which have been clinically evaluated to inhibit CYP19A1 activity include formestane, aminoglutethimide, anastrozole, exemestane, letrozole and testolactone, with anastrozole, exemestane and letrozole being approved in breast cancer patients (Figure 2, Table 1) (Smith and Dowsett, 2003; Hamadeh et al., 2018; Kharb et al., 2020; Ratre et al., 2020; Sood et al., 2021). Molecules such as ketoconazole, anastrozole and letrozole take advantage of a Type II binding mechanism to inhibit their drug targets (Maurelli et al., 2011; Heiday et al., 2021; Mukherjee et al., 2022).

CYP24A1 has generated interest as a potential drug target, given its contributions to the metabolism of 25-hydroxyvitamin D3 and 1α,25-dihydroxyvitamin D3 (Figure 1). Much of the early excitement around CYP24A1 (and CYP27B1 as a prognostic marker) stemmed from their extrahepatic expression in tissues such as the prostate (Chen et al., 2012). While research has been somewhat limited, reports of CYP24A1 inhibitors being used to decrease the metabolism of
endogenous 1α,25-dihydroxyvitamin D3 in an attempt to increase its anti-proliferative and pro-apoptotic effects have been reported, including a number of soy-based natural products (Inouye and Sakaki, 2001; Schuster et al., 2001; McFadyen et al., 2004; Schuster et al., 2006; Bruno and Njar, 2007; Posner et al., 2010; Sheng et al., 2019; Karlsson et al., 2021; Sneha et al., 2021).

Meanwhile, efforts to synthesize inhibitors of CYP26A1, CYP2B1 and CYP26C1 have focused on the enzymes’ roles in multiple diseases, from cancer to dermatological indications (Njar et al., 2006). CYP26 enzyme activity is the primary determinant of both endogenous and exogenously administered levels of all trans-retinoic acid, a downstream product of vitamin A and a key modulator of cell cycle regulation and epithelial cell maintenance through binding to the retinoic acid receptor (Thatcher and Isoherranen, 2009; Stevison et al., 2015; Isoherranen and Zhong, 2019). All-trans retinoic acid is clinically approved in the treatment of acute promyelocytic leukemia, while its isomers 13-cis-retinoic acid and 9-cis-retinoic acid are used to treat both tumors (pediatric neuroblastoma and Kaposi’s sarcoma) and skin disorders (acne and eczema). An entire class of molecules known as retinoic acid metabolism blocking agents (RAMBAs) have been identified and developed with the aim of inhibiting CYP26 activity in vivo. Ketoconazole, liarazole and talarazole have all been evaluated in clinical trials, with efforts ongoing to identify more potent and selective inhibitors of CYP26A1 and CYP26B1 currently underway (Figure 3) (Nelson et al., 2013). The lack of selectivity of the current RAMBAs suggests that additional P450 isoforms may also play a role in retinoic acid homeostasis and pharmacological efficacy.

While P450 isoforms in the CYP1, CYP2 and CYP3 subfamilies are generally more regarded for their roles in xenobiotic metabolism, a number of these enzymes have also been evaluated as possible drug targets. Both CYP1A1 and CYP1B1 have been shown to activate procarcinogens as well as to catalyze the metabolism of estrogen and 17β-estradiol (Napoli et al., 2005). The expression of both enzymes has been shown to be upregulated in multiple cancer types, including head and neck cancer, prostate cancer renal cell carcinoma and lung carcinoma (McFadyen and Murray, 2005; Presa et al., 2021). Further, CYP1A1 and CYP1B1 polymorphisms are associated
with the prevalence of various cancers (Watanabe et al., 2000; Androutsopoulos et al., 2009; Gajjar et al., 2012; Rodriguez and Potter, 2013; Alsubait et al., 2020; Al-Saireh et al., 2021; Yuan et al., 2022). While much of the research surrounding CYP1A1 and CYP1B1 as therapeutic targets has centered on their ability to activate prodrugs, additional approaches including chemical inhibitors, immunotherapy and antisense oligonucleotides have all been described in the literature (McFadyen and Murray, 2005; Luby, 2008; Mescher and Haarmann-Stemmann, 2018; Carrera et al., 2020). Similar to modern T-cell based immunotherapies, a Phase 1 clinical trial was conducted with ZYC300, a vaccine designed to stimulate a CD8+ T-cell response against CYP1B1-expressing tumors (Maecker et al., 2003; Gribben et al., 2005). Further, numerous CYP1B1 inhibitors have been developed and tested preclinically over the years, presumably with the aim of inhibiting procarcinogen activation or in the treatment of various metabolic diseases (Li et al., 2017). Natural products such as flavonoids, coumarins, stilbenes and stilbenoids (ie., resveratrol) have shown antiproliferative effects in vitro, though to date there is limited translation to clinical outcomes (Androutsopoulos et al., 2009; Busbee et al., 2013; Shiiizaki et al., 2017; Alzahrani and Rajendran, 2020). From a prodrug standpoint, clinical trials with Phortress (Figure 4), a lysylamide prodrug of 5F203 (2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole) have taken advantage of CYP1A1 and CYP1B1 tumor expression to activate Phortress, as well as a putative positive feedback loop whereby 5F203 subsequently binds to the arylhydrocarbon receptor (AhR) and induces the expression of CYP1A1 and CYP1B1 (Bradshaw et al., 2002; Fichtner et al., 2004). The increased production of 5F203 was then shown to result in formation of reactive oxygen species, phosphorylation of ERK, JNK and P38/MAPK, resulting in cell cycle arrest and apoptosis in preclinical studies (Bradshaw and Westwell, 2004; Callero et al., 2013). A similar approach has been taken with AFP464 (Figure 4), a lysine prodrug of aminoflavone, though the clinical translation of either Phortress or AFP464 remains limited (Sneha et al., 2021). Finally, series of chemically modified duocarmycin prodrugs such as ITC2700 ( Figure 4) have been designed to specifically target CYP1A1 specifically in tumors, though lack of tumor selectivity has hampered used of these prodrugs in clinical trials (Searcey, 2002; Ghosh et al., 2009; Jukes et al., 2021; Bart et al., 2022).
The catalysis of arachidonic acid epoxidation to epoxyeicosatrienoic acids (EET) by P450 enzymes have led some to postulate the inhibition of this pathway as a potential mechanism to slow cancer metabolism and angiogenesis (Figure 1) (Guo et al., 2018). The overexpression of CYP2C8 and CYP2J2 in various cancer types together with the ability of both of the enzymes to convert arachidonic acid into EETs suggests inhibition of either of these enzymes may subsequently inhibit tumor growth (Dai et al., 2001; Arnold et al., 2017). While not conclusive, the regulation of EET formation by P450s also suggests a possible role for these enzymes in the PI3k/AKT and STAT3 pathways, two mechanisms commonly studied in oncology drug discovery (Spector and Kim, 2015). Mechanism-based inactivation of P450s involved in arachidonic acid epoxidation by drugs such as 17-octadecynoic acid has further supported these claims (Wang et al., 1998; Lin et al., 2017). Building off of this data, inhibitors of CYP2J2 such as telmisartan (angiotensin II receptor antagonist), terfenadine (antihistamine) and C26 (terfenadine analog) and inhibitors of CYP2C8 such as sulfaphenazole have been preclinically tested for disease modifying effects based on inhibition of CYP-mediated EET formation (Figure 5) (Vriens et al., 2005; Chen et al., 2009; Chen et al., 2011; Sisignano et al., 2016). Beyond cancer indications, the CYP2J2 catalyzed formation of EETs has also been researched as a potential anti-inflammatory mechanism as well as one inherently linked to cardiovascular health (Alzahrani and Rajendran, 2020; Aliwarga et al., 2022). In a similar fashion, multiple CYP4 enzymes have been evaluated as possible drug targets owing to their role in HETE (hydroxyeicosatetraenoic acid) formation and fatty acid ω-hydroxylation (Figure 1) (Edson and Rettie, 2013).

With regard to CYP3A, while in general it is considered advantageous to avoid drug interactions with the enzyme given its significant role in the metabolism of a large fraction of currently prescribed drugs, there have been efforts to target the enzyme in order to increase the efficacy of co-administered drugs, as in the case of ritonavir and cobicistat (Tseng et al., 2017). Ritonavir, initially developed as an HIV protease inhibitor, was subsequently found to have properties more in line with boosting the efficacy of co-administered protease inhibitors such as...
atazanavir, darunavir or elvitegravir, by inhibiting hepatic and intestinal CYP3A (and P-glycoprotein) activity (Zeldin and Petruschke, 2004). Ritonavir has a relatively promiscuous drug interaction profile, resulting in the inhibition or induction of multiple P450 and UGT pathways. Cobicistat, on the other hand, is not an HIV protease inhibitor but does improve upon a number of the challenges associated with ritonavir treatment. Similar to ritonavir, cobicistat is a mechanism-based inactivator of CYP3A, but is more selective for CYP3A and thus avoids a number of the unwanted, off-target drug interactions often attributed to ritonavir (Sherman et al., 2015).

**Glutathione S-Transferases**

The observation that GSTs such as GST-π are disproportionally expressed in certain tumor types have led to a significant amount of research over the last 25 years to characterize GSTs as potential pharmacological targets. Attempts to design molecules targeting specific GSTs have followed a similar pattern to much of the P450 work described above. In general, the efforts have fallen into one of three categories, namely inhibitors aimed at decreasing the clearance of chemotherapies that are substrates of GSTs, prodrugs designed to be pharmacologically activated by a specific GST and molecules with the ability to disrupt protein-protein interactions between GSTs and endogenous signaling proteins (Gate and Tew, 2001; Townsend and Tew, 2003; Townsend et al., 2005).

Examples of chemotherapeutic agents that are substrates for GST enzymes include chlorambucil, cisplatin, cyclophosphamide, bleomycin and many of the anthracyclines (Chen and Waxman, 1994; Coles and Kadlubar, 2003). The clinical efficacy of many of these drugs may be hampered by tissue specific glutathione conjugation, catalyzed by various GSTs. Similar to the inhibitory role of ritonavir or cobicistat against CYP3A4 in HIV protease cocktails, co-administration of GST inhibitors with chemotherapy has been explored as a means of increasing the concentration and duration of the drug at the therapeutic site of action (Morgan et al., 1996; Ata and Udenigwe, 2008; Abd El-Karim et al., 2018). Ethacrynic acid (Figure 6), a GST inhibitor commonly used in
ADME phenotyping studies, has been looked at in both preclinical and clinical settings. Using \textit{in vitro} cell lines and \textit{in vivo} tumor models, ethacrynic acid has been shown to increase the tumor killing of drugs such as chlorambucil and melphalan (Tew et al., 1988; Ploemen et al., 1990; Ciaccio et al., 1991; Hansson et al., 1991). Subsequent clinical trials utilizing either chlorambucil or thio-TEPA together with ethacrynic acid have demonstrated positive effects on either drug resistance or pharmacokinetic profiles when the chemotherapies were co-dosed with ethacrynic acid (O'Dwyer et al., 1991; Petrini et al., 1993). Unfortunately, the lack of GST selectivity coupled with the undesirable diuretic properties of ethacrynic acid have limited its use in subsequent chemotherapy regimens (Molnar and Somberg, 2009; Mignani et al., 2016). Sulfasalazine, an inhibitor of GST-\(\alpha\), GST-\(\mu\) and GST-\(\pi\) that is indicated for inflammatory bowel disease, has similarly been shown to increase the efficacy of cisplatin in preclinical and clinical trials (Ma et al., 2015; Otsubo et al., 2017; Shitara et al., 2017a; Shitara et al., 2017b; Thanee et al., 2021). Additional research has focused on peptidic inhibitors of GSTs with the ability to more selectively inhibit a single GST pathway such as TLK-199 or TER-117 (Figure 6), though many of these molecules have suffered from the poor pharmacokinetic properties often associated with peptide-like molecules (Mathew et al., 2006).

The next category of GST-targeted molecules utilizes tissue specific GST activation of a prodrug such as TLK-286 (Telcyta; canfosfamide), metformin- or doxorubicin-like molecules, or nitric oxide producing prodrugs to elicit a therapeutic effect (Allocati et al., 2018). Canfosfamide (Figure 6) is a derivative of glutathione that upon activation by GST-\(\pi\) forms an alkylating agent capable of binding to DNA (Tew, 2005; Dourado et al., 2013). The molecule was evaluated in Phase II and Phase III clinical trials with drug-resistant ovarian cancer patients before ultimately being discontinued for failing to meet its primary endpoints in the Phase III trial. While information is somewhat more limited, series of metformin, doxorubicin or nitric oxide releasing prodrugs have also been developed. Metformin, an oral antidiabetic drug that inhibits mitochondrial complex I, exhibits a high degree of interindividual pharmacokinetic variability (Gong et al., 2012). In an attempt to increase the oral absorption of metformin while also decreasing its inherent variability, a series of
GST-targeting prodrugs were synthesized, taking advantage of GSTs ability to hydrolyze sulfonamide bonds (Ruzza and Calderan, 2013). *In vitro* studies confirmed the increased hydrolysis of these analogs in tissues overexpressing GSTs, however no additional information is available as to whether an improvement in pharmacokinetic variability was observed. Similarly, the ability of GSTs to catalyze sulfonamide hydrolysis was also used in a series of doxorubicin prodrugs, where the rate of cleavage was modified in an attempt to decrease the overall cellular resistance to doxorubicin (van Gisbergen et al., 2016). Finally, nitric oxide releasing prodrugs such as PABA/NO or JS-K result in cellular differentiation and apoptosis through GST-mediated formulation of a diazeniumdiolate anion, followed by spontaneous decomposition and release of nitric oxide resulting in cellular death (Figure 6) (Shami et al., 2003; Saavedra et al., 2006; Chakrapani et al., 2008).

Perhaps the most novel of GST-targeting therapies are those that result in pharmacological action by directly disrupting GSTs' role in maintaining cellular function. As noted above, early work suggested that GST-π inhibits c-Jun N-terminal kinase through specific protein-protein interactions. Beyond c-Jun N-terminal kinase, additional pathways such as NFκB, MAPK and p38 kinases are also affected by this interaction, suggesting the ability of the protein-protein interaction to play a key role in regulating myeloproliferation (LoGrasso et al., 1997; Yin et al., 2000; Dorion et al., 2002; Rodriguez-Ramiro et al., 2012; Jones et al., 2016). For example, TLK199 (and its pharmacologically active moiety, TER117), discussed above as a GST prodrug, was shown to result in myeloproliferation, presumably due to inhibiting the GST-π/c-Jun N-terminal kinase interaction (Ruscoe et al., 2001; Gate et al., 2004; Zhang et al., 2021). The same effect was not observed in GST-π knockout mice.

**UDP-Glucuronosyltransferases**

Beyond P450s and GSTs, other enzyme families primarily known for their drug metabolizing properties have been considered as potential drug targets, although most have yet to show any clinical validation. UGTs, the enzymes responsible for the addition of glucuronic acid and other
sugars to a nucleophilic functional group such as a carboxylic acid, phenol, aliphatic alcohol to many xenobiotics also have a role in the regulation of many endogenous signaling molecules (Tephly and Burchell, 1990; Rowland et al., 2013). For example, various UGTs are involved in the glucuronidation of androgens, estrogens, and lipids, molecules with established roles in tumor growth. Further, numerous groups have shown associations between UGT expression levels and cancer prognoses, suggesting the potential to use UGT profiles as predictive and/or prognostic biomarkers (Desai et al., 2003; Dalhoff et al., 2005; Allain et al., 2020a; Hu et al., 2022). Multiple studies have suggested a role for UGT2B17 in affecting the outcome of chronic lymphocytic leukemia treatments, either due to its role in regulating endogenous steroids or through a non-enzymatic mechanism (Gruber et al., 2013; Bhoi et al., 2016; Allain et al., 2019; Allain et al., 2020b). Similar to the protein-protein interactions described above for GST-π and c-Jun N-terminal kinase, a mechanism for UGT2B17 binding to c-Src and downregulating the downstream, pro-apoptotic Mcl-1 pathway has been proposed (Hirata et al., 2010). To reiterate though, while reports of UGT-targeting therapies remain rare and have not been validated in clinical trials, multiple roles for UGTs in cancer progression and chemotherapeutic drug resistance have been proposed, leaving the door open for subsequent efforts targeting these pathways with modulators of UGT expression or activity.

Outside of oncology, an example of an experimental treatment targeting a UGT isoform involves inhibition of Bm-UGT, an intestinal enzyme present in adult nematodes, as a potential treatment for lymphatic filariasis (Flynn et al., 2019). While the definitive mechanism remains to be elucidated, downregulation of Bm-UGT by siRNA or inhibition by the known UGT inhibitors probenecid or sulfinpyrazone resulted in significant macrofilaricidic effects in vitro, prompting the authors to suggest that further testing may reveal a role for these drugs as clinically relevant treatments for lymphatic filariasis.

**Epoxide Hydrolases**

Epoxide hydrolases are generally grouped into two subfamilies, microsomal epoxide hydrolase (mEH) and soluble epoxide hydrolase (sEH), both of which catalyze the ring-opening of
epoxide-containing molecules to their respective diol products (Seidegard and DePierre, 1983; Hassett et al., 1994; Harris and Hammock, 2013). With regard to drug metabolism, it is primarily the mEHs which contribute to the elimination of xenobiotics and toxic substances including phenytoin, carbamazepine and polyaromatic hydrocarbon intermediates (Gautheron and Jeru, 2020). The sEHs have limited contribution to the metabolism of foreign substances and are more thought of for their role in metabolizing EETs that are produced by P450s in the arachidonic acid pathway, though they do retain the ability to detoxify substances such as terpenoid epoxides (Newman et al., 2005; Imig and Hammock, 2009). With the known anti-inflammatory properties of the various EETs, inhibition of sEH has received a great deal of interest over the past few decades in an attempt to treat multiple diseases, including pain, neurological and cardiometabolic disorders (Schmelzer et al., 2005; Imig and Hammock, 2009; Morisseau and Hammock, 2013; Wagner et al., 2017; Chen et al., 2019; Gowler et al., 2022; Verma et al., 2022). Early attempts at developing sEH inhibitors were based off of glycidols and calcone oxide analogs, two classes of known sEH substrates, and suffered from poor ADME properties due to rapid metabolism by glutathione S-transferases (Imig and Hammock, 2009). Subsequent efforts identified urea-, carbamate- and amide-containing transition state inhibitors of sEH with more drug-like properties (Morisseau et al., 1999; Severson et al., 2002; Hwang et al., 2007). Numerous preclinical studies have demonstrated positive disease-modifying outcomes for sEH inhibitors. For example, sEH knockout mice or mice treated with the sEH inhibitor 1-(1-propionylpiperidin-4-yl)-3-(trifluoromethoxy)phenyl)urea (TPPU; Figure 7) were more resilient and less prone to depressive effects in various stress models, suggesting a role for sEH in treating depression and other social anxiety disorders (Hashimoto, 2016; Ren et al., 2016). Additional neurological indications for sEH inhibitors include Alzheimer’s disease and other neurodegenerative diseases, where compounds that dually inhibit sEH as well as a second pathway (i.e., acetylcholinesterase) have demonstrated protection against neuroinflammation and memory loss (Codony et al., 2022). More recently, the sEH inhibitor EC5026 (Figure 7) has shown promise as non-opioid alternative to the treatment of neuropathic pain, and has advanced into clinical trials (Hammock et al., 2021). From a cardiovascular standpoint, sEH inhibitors have been shown to have
potential roles in lowering blood pressure, reducing vascular inflammation and organ damage, decreasing the risk of cardiac or cerebral ischemia and decreasing weight gain (Chiamvimonvat et al., 2007; Imig and Hammock, 2009; Shen and Hammock, 2012). Unfortunately, most clinical trials with sEH inhibitors have resulted in only mixed success, and generally were regarded as inferior to current treatment regimens. Recently, more interest appears to be on bifunctional inhibitors of sEH and a second pathway as noted above in the Alzheimer’s efforts that coupled inhibition of acetylcholinesterase, with additional targets including PPARγ for renal fibrosis, fatty acid amide hydrolase for chronic pain, and the farnesoid X receptor for the treatment of nonalcoholic steatohepatitis (Schmidt et al., 2017; Kodani et al., 2018; Hye Khan et al., 2019; Schierle et al., 2020; Stavniichuk et al., 2020; Wilt et al., 2021).

**Monoamine Oxidases**

Monoamine oxidase A and B (MAO-A and MAO-B) are two flavin-containing enzymes found in mammals which are known for their role in converting amine-containing molecules to their corresponding aldehyde metabolites (Schnaitman et al., 1967; Kanazawa, 1994). Molecules which are known substrates for MAO enzymes include sumatriptan, almotriptan and other anti-migraine triptans, epinephrine and 5-hydroxytryptamine (MAO-A) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), β-phenylethylamine and benzylamine (MAO-B) (Foti and Dalvie, 2016). As both MAOs are expressed in brain tissue, they play an important role in the homeostatic regulation of key neurotransmitters such as dopamine, serotonin and norepinephrine (Thorpe et al., 1987; Bortolato et al., 2008). To this end, MAOs have long been a key drug target for neurological disorders such as depression, anxiety, bipolar disorder and potentially early Parkinson’s disease (Zornberg and Pope, 1993; Fiedorowicz and Swartz, 2004; Youdim et al., 2004; Youdim and Riederer, 2004; Edmondson and Binda, 2018). Multiple MAO inhibitors have been approved for the aforementioned indications, including isocarboxazid, moclobemide, phenelzine, rasagiline, selegiline, and tranylcypromine (Figure 8, Table 1). The mechanism of inhibition is either irreversible and nonselective (isocarboxazide, phenelzine, tranylcypromine) or reversible and selective.
(moclobemide, selegiline and rasagiline) (Liebowitz et al., 1990). As with mechanism-based inactivation of any drug metabolizing enzyme, the effects of the irreversible inhibitors will linger until new enzyme is resynthesized and as such the pharmacodynamic effects of MAO inhibitors can last for a significant amount of time after stopping treatment.

**Aldo-Keto Reductases**

The reduction of xenobiotic and endogenous aldehydes and ketones to their corresponding alcohols is catalyzed in part by the NADPH dependent superfamily of aldo-keto reductase (AKR) enzymes (Jez et al., 1997; Jin and Penning, 2007; Penning, 2015). The family of AKRs has a wide ranging substrate specificity and is capable of reducing sugars, aldehydes, steroids, prostaglandins, and various carcinogens (Bohren et al., 1989; Penning and Drury, 2007; Spite et al., 2007). AKRs contribute to the drug metabolism of molecules such as oracin, haloperidol, ketotifin and warfarin (Eyles and Pond, 1992; Breyer-Pfaff and Nill, 2000; Wsol et al., 2007; Barnette et al., 2017). Perhaps more important is the role of AKRs in homeostasis and stress responses, contributing to the regulation of glucose, all-trans-retinaldehyde, progesterone analogs and androgens such as 5α- and 5β-dihydrotestosterone (Petrash, 2004; Penning and Byrns, 2009; Hevir et al., 2011; Ruiz et al., 2012; Penning et al., 2019). As such, pharmacological inhibition of AKRs has the potential to have a clinical effect in cardiometabolic diseases, retinopathies, hormonal imbalances and tumor growth. Indeed, a number of clinically approved AKR inhibitors are already in use with additional research to identify new and more effective AKR inhibitors ongoing (Table 1). For example, drugs such as epalrestat, fidarestat, ranirestat, sorbinil and tolrestat (Figure 9) are inhibitors of AKR1B1 and have been used clinically to reduce the onset of diabetic co-morbidities such as retinopathy, cataract formation and neuropathy, albeit it with varying results (Liu et al., 2009; Penning, 2015). An additional example of AKR inhibitors in the clinical setting is 6-methoxyprogesterone acetate (Figure...
9) and other AKR1C3 inhibitors, which have been used in the treatment of acute myeloid leukemia and other cancers where the reduction of 17β-hydroxysteroid dehydrogenase activity would be seen as advantageous (Penning, 2015). Additional examples of preclinical research efforts aimed at AKR inhibition can be found in a number of excellent recent reviews (Chang and Petrash, 2018; Khayami et al., 2020; Liu et al., 2020; Penning et al., 2021).

**Xanthine Oxidase**

Finally, aldehyde oxidase (AO) and xanthine oxidase (XO) are molybdenum hydrolases that are expressed cytosolically (Linder et al., 1999; Garattini and Terao, 2012). As medicinal chemistry efforts to decrease P450-mediated clearance continue to be refined, the contribution of both enzymes to the overall metabolism of xenobiotics is expected to increase. Both AO and XO are capable of oxidizing aldehydes and aromatic heterocycles and while the exact physiological function of AO remains unknown, there is evidence that XO plays a role in the regulation of purine base metabolism, namely the conversion of hypoxanthine to uric acid (Krenitsky et al., 1972; Panoutsopoulos et al., 2004; Alfarro and Jones, 2008; Pryde et al., 2010). As the generation of reactive oxygen species is a byproduct of hypoxanthine metabolism, the inhibition of XO has received attention as a potential drug target to reduce the amount of reactive oxygen species, increase nitric oxide production and ameliorate endothelial dysfunction and improve cardiovascular health (Bredemeier et al., 2018). Inhibitors of xanthine oxidase are divided into either purine-like or nonpurine-like classes (Figure 10, Table 1). Allopurinol is a purine-like molecule that is indicated for the treatment of gout, recurring kidney stones and chemotherapy-induced increases in uric acid levels (Massey et al., 1970; Pacher et al., 2006). Allopurinol inhibits XO in a pseudo-irreversible mechanism, with its main metabolite, oxypurinol, being a potent inhibitor of XO both in the presence and absence of allopurinol (Spector, 1977). Febuxostat and topiroxostat (approved in Japan) are nonpurine-like molecules that are prescribed for the treatment chronic hyperuricemia in patients who
do not respond to or demonstrate intolerance to allopurinol (Cicero et al., 2021). Unlike allopurinol, febuxostat and topiroxostat block enzyme turnover of XO through the formation of a stable enzyme-inhibitor complex, irrespective of the oxidation state of the molybdenum cofactor (Okamoto et al., 2003). Preclinically, potent time-dependent inhibitors of XO such as 3,4-dihydroxy-5-nitrobenzaldehyde have also been evaluated for pharmacological activity (Lu et al., 2013).

**Current Challenges and Knowledge Gaps**

As would be expected for the development of a drug against any given pharmacological target, the discovery and characterization of drugs against enzymatic targets which have evolutionarily evolved to eliminate foreign substances is fraught with key challenges as well (Foti et al., 2011). Given the primary role of these enzymes, perhaps the most obvious challenge is identifying an inhibitor which is not a readily metabolized substrate of either the target or any closely related enzymes, resulting in a rapid clearance of the drug *in vivo*. While target-mediated drug disposition (TMDD) is a concept most often thought of with protein therapeutics, examples of TMDD for small molecules have also been noted, such as with the DPP-IV inhibitors vildagliptin and AMG 222, where target-mediated hydrolysis of a cyanopyrrolidine moiety to the corresponding acid or amide metabolites is considered to be a major pathway in the disposition of both molecules (He et al., 2009; Greene et al., 2011). Conversely, small molecule TMDD can be the result of a very potent inhibitor that exhibits tight binding kinetics to its target, resulting in a nonlinear pharmacokinetic profile for the inhibitor, as the fraction of the molecule that is bound to target is not available to be cleared from circulation (An, 2020). Such TMDD profiles have been reported as challenges for series of sEH inhibitors and 11β-hydroxysteroid dehydrogenase inhibitors, as well as the DPP-IV inhibitors noted above (An et al., 2015; An et al., 2021).

Following efforts to reduce enzymatic clearance, the avoidance of drug-drug interactions is another common aspect of the ADME focus early in drug discovery. For more “traditional” drug
targets, counter-screens are commonly run to ensure selectivity for the target of interest in order to avoid unwanted, off-target pharmacology (Hughes et al., 2011). From a selectivity standpoint, proteins whose active sites share a high degree of homology can often prove problematic, especially when similar physicochemical properties govern ligand binding interactions across a series of closely related proteins. For example, as noted above, many of the inhibitors of CYP17A1, CYP19A1 or GSTπ were limited in their clinical utility owing to non-selective inhibition of multiple P450 or GST isoforms, including hepatic isoforms involved in the clearance of xenobiotics (Gate and Tew, 2001; Smith et al., 2002). Similarly, substrate overlap resulting in multiple enzymes being responsible for the regulation of a given endogenous pathway (ie., numerous P450s involved in arachidonic acid metabolism) further complicates the possibility of finding a selective and pharmacodynamically active molecule against such a pathway (Capdevila et al., 2000).

An additional knowledge gap which has received a considerable amount of attention in recent years, especially with advancements in proteomics workflows, is the amount of information surrounding tissue- and tumor-specific expression patterns of drug metabolizing enzymes (Kural et al., 2019; Nekvindova et al., 2020; Zhao et al., 2020). In addition to protein quantitation, the ability to better estimate the fraction of a given pathway that is controlled by a given enzyme plays a determining factor in which drug target to pursue for a given pharmacological outcome (ie., P450 versus cyclooxygenase versus lipoxygenase contributions to arachidonic acid metabolism). Indeed, all of the P450 contributions noted in Figure 1 do not necessarily indicate the primary determining factors in a given pathway. Further, for prodrugs that utilize overexpression of a given enzyme in a specific tissue to avoid unwanted toxicities in healthy tissue, a better understanding of the expression, regulation and function of these enzymes, and perhaps equally as important, in vitro systems capable of providing such information, will be key (Sneha et al., 2021).

**Conclusion and Perspective on Future Directions**
While much of the research focused on the enzymes covered in this minireview has been from an ADME standpoint characterizing their role in metabolizing xenobiotics and toxic substances, the additional role of drug metabolizing enzymes in homeostasis and the regulation of endogenous signaling molecules has made them attractive as potential drug targets. Indeed, significant and lasting contributions have been made towards understanding the mechanisms of the P450, GST, sEH, AKR and other families of enzymes in activating or deactivating biological pathways involved in tumorigenesis, inflammation, cardiovascular health and neurodegenerative diseases. As the roles of additional enzymes such as the carboxylesterases, arylacetamide deacetylase and other serine hydrolases in both drug metabolism and endogenous regulation become more understood, it is possible that similar efforts to evaluate these enzymes as drug targets will be undertaken (Redinbo and Potter, 2005; Dominguez et al., 2014; Shimizu et al., 2014; Wang et al., 2018; Jin et al., 2022a; Jin et al., 2022b). Of key importance in developing direct inhibitors of drug metabolizing enzymes as pharmaceuticals will be continuing to improve our knowledge of the importance of a given enzymatic contribution (ie., fraction metabolized) to the overall regulation of a signaling pathway relative to other regulatory mechanisms, coupled with well-designed experiments aimed at comparing inhibition of part of a pathway (ie., inhibiting estrogen synthesis) vs the entire pathway (ie., blocking the estrogen receptor). Further, for inhibitors or prodrugs which aim to capitalize on tissue specific expression patterns of enzymes, continuing to solidify our knowledge base of those expression patterns and how they change with given disease states is crucial. Finally, while selective, stand-alone therapeutics against drug metabolizing enzymes may continue to prove challenging, the possibility of combining enzyme inhibitors or inducers with other treatments to enhance the efficacy of the co-administered treatment should serve to combine the core strengths of ADME and pharmacology towards bringing more safe and effective treatment options to patients.
**Authorship Contributions**

*Participated in research design:*

*Conducted experiments:*

*Contributed new reagents or analytical tools:*

*Performed data analysis:*

*Wrote or contributed to the writing of the manuscript: Foti*
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Edson KZ and Rettie AE (2013) CYP4 enzymes as potential drug targets: focus on enzyme multiplicity, inducers and inhibitors, and therapeutic modulation of 20-


Footnotes

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Figure Legends

Figure 1. Contribution of cytochrome P450 and other drug metabolizing enzymes to vitamin D3, cholesterol, steroid and arachidonic acid homeostasis. Inclusion of an enzyme in a given pathway simply denotes an experimentally demonstrated contribution of that enzyme to the pathway and should not be assumed that it is the major or only determinant of regulating a given pathway. Information collated from (Pikuleva, 2006; Chiamvimonvat et al., 2007; Imig and Hammock, 2009; Chen et al., 2012; Shen and Hammock, 2012; Pikuleva and Waterman, 2013; Rowland and Mangoni, 2014; El-Sherbeni and El-Kadi, 2017) and references therein.

Figure 2. Inhibitors of CYP17A1 (abiraterone, ketoconazole) or CYP19A1 (formestane, aminoglutethimide, anastrozole, exemestane, letrozole, testolactone).

Figure 3. Examples of Retinoic Acid Metabolism Blocking Agents (RAMBAs). Inhibition of CYP26A1- and CYP26B1-catalyzed retinoic acid by RAMBAs is aimed at increased concentrations of endogenous or exogenous retinoic acid. Similar to CYP17A1 and CYP19A1, many RAMBAs suffer from a lack of selectivity for CYP26A1 or CYP26B1.

Figure 4. CYP1A-activated prodrugs Phortress, AFP464 and ITC2700 (duocarmycin analog). Initial experiments targeted tumor-selective activation and/or induction of CYP1A by AhR (Phortress and AFP464), though clinical translation has been limited.

Figure 5. Molecules evaluated preclinically for inhibition of CYP2J2-catalyzed epoxideicosatrienoic acid formation. 17-Octadecynoic acid (P450 mechanism-based inactivator), telmisartan (angiotensin II receptor antagonist) and terfenadine or C26 (antihistamines) have demonstrated disease-modifying effects in preclinical models due to the reduction of EET formation.

Figure 6. Inhibitors of glutathione S-transferase. Preclinical and clinical attempts to inhibit GST activity have utilized inhibitors (ethacrynic acid and its GSH conjugate), prodrugs to overcome poor pharmacokinetics limitations (TLK-199) and GST-mediated activation (canfosfamide, PABA/NO).

Figure 7. Soluble epoxide hydrolase (sEH) inhibitors 1-(1-propionylpiperidin-4-yl)-3-(trifluoromethoxy)phenyl)urea (TPPU) and EC5026. Rationale synthesis of molecules like EC5026 were aimed at improving upon ADME and pharmaceutical properties of earlier sEH inhibitors for reduction of pain and inflammation.

Figure 8. Inhibitors of monamine oxidase (MAO-A and MAO-B). Irreversible inhibitors of MAO (isocarboxazide, phenelzine and tranylcypromine) inactivate both MAO isoforms through a covalent mechanism requiring resynthesis of new enzyme before activity is restored. Selective and reversible inhibitors target either MAO-A (moclobemide) or MAO-B (selegiline, rasagiline) utilizing a competitive inhibition mechanism.

Figure 9. Aldo-ketoreductase inhibitors utilized in the treatment of cardiometabolic diseases or acute myeloid leukemia. Inhibitors of AKR1B1 (epalrestat, fidarestat, ranirestat, sorbinil, tolrestat) have been evaluated clinically for the reduction of diabetic comorbidities. 6-Medroxyprogesterone acetate (AKR1C3 inhibitor) has been used as adjuvant therapy in the treatment of acute myeloid leukemia and other cancer types.

Figure 10. Xanthine oxidase inhibitors. Inhibitors of XO are classified as purine-like (allopurinol) or nonpurine-like (febuxostat, topiroxostat) and are used in the treatment of hyperuricemia-related diseases.
## Table 1

Examples of Approved Drugs Targeting Metabolic Enzymes in the Treatment of Various Diseases (Drugs with irreversible mechanisms of action are denoted with an asterisk).

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Target Enzyme</th>
<th>Primary Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobicistat*</td>
<td>CYP3A</td>
<td>Pharmacokinetic booster in HIV combination treatments</td>
</tr>
<tr>
<td>Mitotane</td>
<td>CYP11A1 / CYP11B1</td>
<td>Adrenal cortical cancer</td>
</tr>
<tr>
<td>Aminoglutethimide</td>
<td>CYP11A1 / CYP19A1</td>
<td>Cushing’s Syndrome</td>
</tr>
<tr>
<td>Abiraterone acetate</td>
<td>CYP17A1</td>
<td>Castration resistant prostate cancer</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>CYP17A1</td>
<td>Castration resistant prostate cancer</td>
</tr>
<tr>
<td>Anastrozole</td>
<td>CYP19A1</td>
<td>HR+ breast cancer</td>
</tr>
<tr>
<td>Exemestane*</td>
<td>CYP19A1</td>
<td>Adjuvant therapy in ER+ breast cancer</td>
</tr>
<tr>
<td>Letrozole</td>
<td>CYP19A1</td>
<td>HR+ breast cancer</td>
</tr>
<tr>
<td>Testolactone</td>
<td>CYP19A1</td>
<td>Advanced breast cancer</td>
</tr>
<tr>
<td>Formestane*</td>
<td>CYP19A1</td>
<td>Adjuvant therapy in ER+ breast cancer</td>
</tr>
<tr>
<td>Moclobemide</td>
<td>MAO-A</td>
<td>Major depressive disorder, bipolar disorder</td>
</tr>
<tr>
<td>Rasagiline*</td>
<td>MAO-B</td>
<td>Parkinson’s Disease</td>
</tr>
<tr>
<td>Selegiline*</td>
<td>MAO-B</td>
<td>Parkinson’s Disease</td>
</tr>
<tr>
<td>Isocarboxazid*</td>
<td>MAO-A / MAO-B</td>
<td>Treatment resistant depression</td>
</tr>
<tr>
<td>Phenelzine*</td>
<td>MAO-A / MAO-B</td>
<td>Treatment resistant depression, panic disorder, social anxiety disorder</td>
</tr>
<tr>
<td>Tranylcypromine*</td>
<td>MAO-A / MAO-B</td>
<td>Major depressive disorder</td>
</tr>
<tr>
<td>Epalrestat</td>
<td>AKR1B1</td>
<td>Diabetic neuropathy (Japan)</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Xanthine Oxidase</td>
<td>Hyperuricemia</td>
</tr>
<tr>
<td>Febsuxostat</td>
<td>Xanthine Oxidase</td>
<td>Allopurinol-resistant hyperuricemia</td>
</tr>
<tr>
<td>Tepiroxostat</td>
<td>Xanthine Oxidase</td>
<td>Hyperuricemia (Japan)</td>
</tr>
</tbody>
</table>
Figure 1

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Figure 2

Abiraterone Acetate

Ketoconazole

Formestane

Aminogluthethimide

Anastrolzole

Exemestane

Letrozole

Testolactone
Figure 4

Phortress

\[ \text{O} \]
\[ \text{NH} \]
\[ \text{N} \]
\[ \text{FS} \]
\[ \text{H}_2\text{N} \]
\[ \text{H}_2\text{N} \]
\[ \text{H}_2\text{N} \]
\[ \text{H}_2\text{N} \]

5F203

\[ \text{O} \]
\[ \text{NH} \]
\[ \text{O} \]
\[ \text{F} \]
\[ \text{F} \]
\[ \text{F} \]
\[ \text{F} \]

Aminoflavone

AFP464

\[ \text{O} \]
\[ \text{NH} \]
\[ \text{O} \]
\[ \text{F} \]
\[ \text{F} \]
\[ \text{F} \]
\[ \text{F} \]

ITC2700

\[ \text{O} \]
\[ \text{NH} \]
\[ \text{O} \]
\[ \text{F} \]
\[ \text{F} \]

Spirocyclization

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Figure 6

Ethacrynic Acid

Canfosfamide

Ezatiostat (TLK-199)

TER-177

PABA / NO

DMD Fast Forward. Published on April 11, 2023 as DOI: 10.1124/dmd.122.001011
Irreversible and Nonselective

Isocarboxazide

Phenelzine

Tranylcypromine

Reversible and Selective

Moclobemide

Selegiline

Rasagiline
Figure 10

![Chemical structures of Allopurinol, Febuxostat, and Topiroxostat]