INTRODUCTION TO DRUG-DRUG INTERACTIONS

Drug-drug interactions are a major cause of hospitalization and deaths related to drug use. A large fraction of these is due to inhibition of enzymes involved in drug metabolism and transport, particularly cytochrome P450 (P450) enzymes. Understanding basic mechanisms of enzyme inhibition is important, particularly in terms of reversibility and the use of the appropriate parameters. In addition to drug-drug interactions, issues have involved interactions of drugs with foods and natural products related to P450 enzymes. Predicting drug-drug interactions is a major effort in drug development in the pharmaceutical industry and regulatory agencies. With appropriate in vitro experiments, it is possible to stratify clinical drug-drug interaction studies. A better understanding of drug interactions and training of physicians and pharmacists has developed. Finally, some P450s have been the targets of drugs in some cancers and other disease states.

INTRODUCTION TO DRUG-DRUG INTERACTIONS

Drug-drug interactions are important and accordingly are a major issue in the advancement of new chemical entities to patients. In one study (Montané et al., 2018), drug-related deaths accounted for 7% of all deaths in hospital settings. Drug-drug interactions are an issue in this regard. Although some pharmacodynamic issues are involved, a large fraction of the drug-drug interactions is pharmacokinetic in nature. A general overview of the interactions of a chemical with an enzyme is shown in Fig. 1A, where an enzyme involved in the metabolism of a drug is considered. The drug is converted to a product, often called a metabolite. With respect to the parent drug molecule, the product may have unaltered pharmacological activity, lose some or all of its pharmacological activity, be even more active, or be toxic. In the interactions of two drugs, one is sometimes termed the “perpetrator” and one the “victim” (Fig. 1B). In some cases, drug interaction due to enhanced metabolism by induction or allosteric activation may be seen and have clinical consequences (Bott et al., 1977), but the focus here will be on competitive and irreversible inhibitors that attenuate drug metabolism (as “perpetrators”).

Recently Yu et al. (2018) evaluated drug-drug interactions in 103 recent drug approvals by the United States Food and Drug Administration (FDA). Of these, 45 were involved as victims (substrates) in interactions with marketed drugs (perpetrators). The therapeutic classes are shown in Fig. 2A, with cancer treatments accounting for more than 1/4. The enzymes involved are primarily cytochrome P450 and transporter enzymes (Fig. 2B), with P450 3A4/5 involved in ~2/3 of the interactions. Of the 103 new drugs, 20 were acting as perpetrators to some extent (Yu et al., 2018), with P450 3A4/5 and P-glycoprotein again being the most prominent enzymes (Fig. 1B). Accordingly, this review will focus on issues of inhibition of P450 3A4 (In general P450 3A4 and 3A5 have similar substrate specificity and are sensitive to the same inhibitors, with some important exceptions (Hardy et al., 2014; Zhu et al., 2014; Kramlinger et al., 2015), but many experiments with drugs were done only with P450 3A4—or 3A4 and 3A5 were not discriminated—and will be referred to as 3A4 in that context).

BACKGROUND ON P450S

P450s enzymes are the main catalysts involved in the oxidation of chemicals in general (Rendic and Guengerich, 2015), including drugs. The same is true for involvement in
steroid metabolism (Auchus and Miller, 2015). The history of P450 research can be traced to the 1940s and the interest in the metabolism of drugs, steroids, and carcinogens (Williams, 1947; Mueller and Miller, 1948; Ryan, 1959), but the actual discovery of P450 as an entity developed from biochemical interests in the spectral properties of liver cytochromes (Omura and Sato, 1962, 1964). For the history of the characterization of P450s, elucidation of chemical mechanisms of catalysis, gene regulation, pharmacogenetics, and development of the understanding of roles in drug metabolism and deposition, see (Ortiz de Montellano, 2015; Guengerich, 2019b; Parkinson et al., 2019).

Fig. 1. (A) General scheme for interaction of a drug with an enzyme involved in its metabolism. (B) Interactions of “perpetrator” and “victim” drugs.

Fig. 2. Frequency of new molecular entities (NMEs, i.e. new drug candidates) in inhibition-based drug-drug interactions (DDIs) with drugs approved by the Food and Drug Administration (FDA) in the United States between 2013 and 2016 (Yu et al., 2018). (A) Grouping by therapeutic class. (B) Grouping by enzymes involved. Pgp and OAT1B1 are transporters. COMT: catechol O-methyl transferase.

With the development of the Human Genome Project, it was established that there are 57 human P450 (CYP) genes (Table 1). Whether some of these are expressed at appreciable levels (e.g., 2A7, 3A43) is yet unclear, but there are two splice variants of P450 4F3 expressed, so the number of human P450s is still ~57. Several of the P450s remain largely uncharacterized in terms of function and can be considered “orphans” in the context of a classification of P450s by substrate (Table 1).

Some of the reactions shown to be catalyzed by P450s are slow and may not be indicative of more relevant reactions that the enzyme might be doing (e.g., P450 2S1, 2U1, 4X1) (Fekry et al., 2019). Some P450s could be grouped under multiple headings (e.g., 1B1—steroids (estrogens) and xenobiotics (polycyclic hydrocarbons)). As a corollary, some of the P450s that are recognized for their roles in the metabolism of en-
dogenous compounds can also act on xenobiotic chemicals, including drugs or drug candidates, e.g., P450 11A1 (Zhang et al., 2012).

Five P450s in the xenobiotics column (i.e., 1A2, 2C9, 2C19, 2D6, 3A4) have historically accounted for ~90% of the P450 reactions with drugs, and P450s have been the main enzymes involved in the metabolism of (small molecule) drugs (Rendic and Guengerich, 2015; Bhutani et al., 2021). The overall situation has not changed over the years, but pharmaceutical companies have tried to reduce the fraction of metabolism done by the highly polymorphic P450s, mainly P450s 2D6 and 2C19 (Fig. 3). Perhaps because of this, an even larger fraction of drugs now seems to be metabolized largely by P450 3A4 (Fig. 3). This trend has some consequences in the potential for drug-drug interactions, as described below.

Beginning in the 1980s, it became possible to use in vitro methods to discern which drugs are substrates, inhibitors, and inducers of individual P450s (Guengerich, 1989). Such predic-
tions can be confirmed in humans in vivo in many cases. Of relevance to this review, a list of inhibitors of five major human P450s involved in drug metabolism was prepared by the late Prof. David Flockhart, and a website is maintained at Indiana University (Table 2). This information can be very useful to pharmacists and physicians who prescribe medicine and fill prescriptions (as well as those involved in drug development).

**TYPES OF INHIBITION**

It is useful to review basic information about enzyme in-

![](https://doi.org/10.4062/biomolther.2021.102)

**Table 2. Inhibitors of major P450s**

| 1A2      | 2C9      | 2C19     | 2D6      | 3A4      |
|----------|----------|----------|----------|----------|
| Amiodarone | Amiodarone | Chloramphenicol | Amiodarone | Amiodarone |
| Cimetidine | Clopidogrel | Cimetidine | Bupropion | Aprepitant |
| Ciprofloxacin | Clopidogrel | Citalopram | Celecoxib | Atomoxetine |
| Citalopram | Crisaborole | Esomeprazole | Chlorpheniramine | Boceprevir |
| Crisaborole | Efavirenz | Felbamate | Chlorpromazine | Chloramphenicol |
| Efavirenz | Fenofibrate | Fluoxetine | Cimetidine | Cimetidine |
| Fluoroquinolone | Fluconazole | Fluvoxamine | Cinacalcet | Ciprofloxacin |
| Fluvoxamine | Fluoxastatin | Indomethacin | Citalopram | Clarithromycin |
| Furafylline | Fluoxastamine | Isoniazid | Clemastine | Delavirdine |
| Interferon | Isoniazid | Ketoconazole | Clomipramine | Diethylthiocarbamate |
| Methoxsalen | Lovastatin | Lansoprazole | Cocaine | Diltiazem |
| Mibefradil | Metronidazole | Modafinil | Diphenhydramine | Erythromycin |
| Ribocilb | Paroxetine | Omeprazole | Doxepin | Esomeprazole |
| Rucaparib | Phenylbutazone | Oxcarbazepine | Doxorubicin | Fluconazole |
| Ticlopidine | Probenidic | Pantoprazole | Duloxetine | Fluvoxamine |
| Rucaparib | Probenidic | Pantoprazole | Duloxetine | Fluvoxamine |
| Sertraline | Rucaparib | Probenidic | Escitalopram | Gledosate |
| Sulfamethoxazole | Ticlopidine | Halofantrine | Piroxicam | Ibrutinib |
| Sulfaphenazole | Ropiramate | Haloperidol | Ibrutinib |
| Teniposide | Voriconazole | Hydroxyzine | Indinavir |
| Voriconazole | Voriconazole | Hydroxyzine | Ibrutinib |
| Zafirlukast | Methadone | Ketoconazole | Ibrutinib |
| Amiodarone | Amiodarone | Chloramphenicol | Amiodarone | Amiodarone |
| Cimetidine | Clopidogrel | Cimetidine | Bupropion | Aprepitant |
| Ciprofloxacin | Clopidogrel | Citalopram | Celecoxib | Atomoxetine |
| Citalopram | Crisaborole | Esomeprazole | Chlorpheniramine | Boceprevir |
| Crisaborole | Efavirenz | Felbamate | Chlorpromazine | Chloramphenicol |
| Efavirenz | Fenofibrate | Fluoxetine | Cimetidine | Cimetidine |
| Fluoroquinolone | Fluconazole | Fluvoxamine | Cinacalcet | Ciprofloxacin |
| Fluvoxamine | Fluoxastatin | Indomethacin | Citalopram | Clarithromycin |
| Furafylline | Fluoxastamine | Isoniazid | Clemastine | Delavirdine |
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| Methoxsalen | Lovastatin | Lansoprazole | Cocaine | Diltiazem |
| Mibefradil | Metronidazole | Modafinil | Diphenhydramine | Erythromycin |
| Ribocilb | Paroxetine | Omeprazole | Doxepin | Esomeprazole |
| Rucaparib | Phenylbutazone | Oxcarbazepine | Doxorubicin | Fluconazole |
| Ticlopidine | Probenidic | Pantoprazole | Duloxetine | Fluvoxamine |
| Rucaparib | Probenidic | Pantoprazole | Duloxetine | Fluvoxamine |
| Sertraline | Rucaparib | Probenidic | Escitalopram | Gledosate |
| Sulfamethoxazole | Ticlopidine | Halofantrine | Piroxicam | Ibrutinib |
| Sulfaphenazole | Ropiramate | Haloperidol | Ibrutinib |
| Teniposide | Voriconazole | Hydroxyzine | Indinavir |
| Voriconazole | Voriconazole | Hydroxyzine | Ibrutinib |
| Zafirlukast | Methadone | Ketoconazole | Ibrutinib |
| Amiodarone | Amiodarone | Chloramphenicol | Amiodarone | Amiodarone |
| Cimetidine | Clopidogrel | Cimetidine | Bupropion | Aprepitant |
| Ciprofloxacin | Clopidogrel | Citalopram | Celecoxib | Atomoxetine |
| Citalopram | Crisaborole | Esomeprazole | Chlorpheniramine | Boceprevir |
| Crisaborole | Efavirenz | Felbamate | Chlorpromazine | Chloramphenicol |
| Efavirenz | Fenofibrate | Fluoxetine | Cimetidine | Cimetidine |
| Fluoroquinolone | Fluconazole | Fluvoxamine | Cinacalcet | Ciprofloxacin |
| Fluvoxamine | Fluoxastatin | Indomethacin | Citalopram | Clarithromycin |
| Furafylline | Fluoxastamine | Isoniazid | Clemastine | Delavirdine |
| Interferon | Isoniazid | Ketoconazole | Clomipramine | Diethylthiocarbamate |
| Methoxsalen | Lovastatin | Lansoprazole | Cocaine | Diltiazem |
| Mibefradil | Metronidazole | Modafinil | Diphenhydramine | Erythromycin |
| Ribocilb | Paroxetine | Omeprazole | Doxepin | Esomeprazole |
| Rucaparib | Phenylbutazone | Oxcarbazepine | Doxorubicin | Fluconazole |
| Ticlopidine | Probenidic | Pantoprazole | Duloxetine | Fluvoxamine |
| Rucaparib | Probenidic | Pantoprazole | Duloxetine | Fluvoxamine |
| Sertraline | Rucaparib | Probenidic | Escitalopram | Gledosate |
| Sulfamethoxazole | Ticlopidine | Halofantrine | Piroxicam | Ibrutinib |
| Sulfaphenazole | Ropiramate | Haloperidol | Ibrutinib |
| Teniposide | Voriconazole | Hydroxyzine | Indinavir |
| Voriconazole | Voriconazole | Hydroxyzine | Ibrutinib |
| Zafirlukast | Methadone | Ketoconazole | Ibrutinib |

Modified from Flockhart, D. A. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine (2007). “https://drug-interactions.medicine.iu.edu” Accessed 27 August 2021 (Flockhart, 2007).
tion of parameters ($k_{\text{cat}}$, $K_m$, $k_{\text{cat}}/K_m$) because they are based on in appropriate weighting of data obtained with low substrate concentrations (Johnson, 2019). Second, these are idealized situations and may well not reveal enzyme complexity. For instance, the inhibition of P450-catalyzed oxidation of nifedipine or quinidine by cholesterol appears to follow non-competitive kinetics but it is known that cholesterol itself is a substrate (4β-hydroxylation) (Shinkyo and Guengerich, 2011). That is, cholesterol is both a substrate and an inhibitor. An explanation is that the large active site of P450 3A4 allows both occupancy by both cholesterol and another substrate, with cholesterol either binding in the substrate site or elsewhere nearby (Fig. 5). This is an adaptation of a more general mechanism proposed by Segel (1975). Finally, “mixed” inhibition is involved when the intercept of the two lines (no inhibitor, plus inhibitor) in Fig. 4 is to the left of the x=0 axis but not on the y=0 axis. This is generally assumed to mean that neither of the two reactions shown for binding the inhibitor I in Fig. 4B is predominating, i.e. binding to free enzyme (E) or the enzyme-substrate complex (ES). However, the mechanism may be more complex and more detailed analysis is probably in order. Steady-state methods are really only a prelude into mechanistic studies, and pre-steady-state approaches are more powerful if they can be applied (Johnson, 2019).

Table 3. Types of Inhibition

| Type of Inhibition |
|--------------------|
| Reversible         |
| Competitive        |
| Non-competitive    |
| Uncompetitive      |
| Mixed              |
| Time-dependent (“irreversible”) |
| Formation of inhibitory product |
| Electrophile or radical |
| ROS                |
| Metabolite complex (-N=O or C:) (nitroso or carbene) |
| Mechanism-based    |
| Slow, tight-binding |

Competitive inhibition can often be problematic. For instance, kinetic simulations clearly show that the order of addition of substrate and inhibitors can change the apparent outcome inhibition constant ($K_i$), and the effect of the order is more pronounced with a strong inhibitor or with time-dependent inhibition (Guengerich, 2019a). Substrate depletion can alter parameters ($K_m$) and even generate apparently sigmoidal plots. A rate-limiting step following product formation lowers the apparent $K_m$ and also distorts the observed $K_i$. The consumption of an inhibitor during a reaction affects the apparent $K_i$, the extent of which differs depending on which enzyme is involved—the target or another enzyme consuming the inhibitor.

In contrast to reversible inhibition, irreversible inhibition reactions are time-dependent and are of several types (Table 3). In one case, a P450 generates a reactive product that can react with that P450 and perhaps with other molecules as well. One example is chloramphenicol, where the hydroxylation of a -CHCl moiety yields a gem-halohydrin (-CH(OL)Cl) and then an acyl chloride (-C(O)Cl), which reacts with lysines (Halpert and Neal, 1980). In this regard, one can also consider prod-
ucts of oxygen to be inhibitory products, i.e. reactive oxygen species (O$_2$•$^-$, H$_2$O$_2$). Some P450s (e.g., 4A11) appear to be very sensitive to this phenomenon, with conversion of the thiolate group normally liganded to the heme being oxidized to a sulfenic acid (Albertolle et al., 2017, 2018, 2019).

A special case is the production of C-nitroso and carbene products, where the product binds tightly to the heme iron (in its ferrous form). Sometimes this phenomenon has been termed “metabolite inhibition” (complexation). The most common cases where this happens are with primary amines (which may be generated from secondary or tertiary amines) and methylenedioxyphenyl compounds that yield carbenes. These complexes are recognized by their characteristic Soret spectra at 455 nm that form during the reactions (Franklin and Buening, 1974; Mansuy et al., 1979; Paulsen-Sörman et al., 1984).

Another type of time-dependent irreversible inhibition is true mechanism-based inactivation (Fig. 6). This is distinguished from the generation of reactive products in that a reactive entity is generated in the course of the reaction but does not leave the enzyme (Abeles and Maycock, 1976; Silverman, 1995). Such inhibition, in contrast to generation of reactive products, is distinguished by the lack of attenuation by nucleophilic scavengers, e.g. glutathione. In many cases the products of the reaction with the P450 protein (or its heme prosthetic group) have been identified (Correia and Hollenberg, 2015; Lin et al., 2018). An important example is bergamottin, a component of grapefruit juice responsible for interaction with P450 3A4 (Bjornsson et al., 2003a, 2003b; Shou and Dai, 2008). Thus, most pharmaceutical companies use assays with FDA-recommended substrates, e.g. phenacetin for P450 1A2, diclofenac for P450 2C9, (S)-mephentoin for P450 2C19, dextromethorphan for P450 2D6, and testosterone and midazolam for P450 3A4 (Shou and Dai, 2008; Alyamani et al., 2017). These assays usually involve HPLC and mass spectrometry. In general, the recommended substrate concentrations to use are near the $K_m$ values. Positive controls (with accepted inhibitors) should be done at concentrations low enough to be selective.

Typically, a battery for testing inhibition would be done in the order shown in Table 4, with the scientific content of the results—and the cost—increasing at each step. A $K_i$ is superior to an IC$_{50}$ in that the IC$_{50}$ value will be dependent upon the substrate concentration, but a $K_i$ is not.

Time-dependent inhibition is problematic, for several reasons. This phenomenon gives rise to varying pharmacokinetics and is difficult to model, because of the issue of the time needed to synthesize new protein in vivo. The effects of repeated doses are hard to model.

Zimmerlin et al. (2011) surveyed Novartis drugs on the market; only 4% showed time-dependent inhibition, and another 3% showed strong but reversible inhibition (Fig. 7). However, 23% of “new chemical entities” (under development) showed...
time-dependent inhibition and 9% were strong but reversible inhibitors (Zimmerlin et al., 2011), implying that strong and time-dependent inhibitors tend not to survive through the development process and get to market. A further analysis of time-dependent inhibitory drugs in shown in Fig. 8, ordered by the rate of inactivation. Although the mechanisms of some of these are known (e.g., 17α-ethynylestradiol (EE2), gestodene, troleandomycin), in other cases the chemistry underlying the inhibition is not very obvious. Similar experience at Pfizer has been reported (Fig. 9), with the incidence of in vivo time-dependent P450 3A4 inhibition being as high as 75% among candidates in many programs (Eng et al., 2021). As in the case of the Novartis compounds (Zimmerlin et al., 2011), many of these do not have chemical features typical of mechanism-based inactivation. The incidence of time-dependent inhibition was less in human hepatocytes than in liver microsomes, for reasons that are yet unknown. Even among the drugs that were time-dependent inhibitors of P450 3A4 in hepatocytes and in vivo, the structural reasons remain unclear.

Work with P450 3A4 in this laboratory has shown that the
interactions of many inhibitors with the enzyme is a multi-step process, as judged by the appearance of multiple spectral species over a period of up to 20 seconds or more (Guengerich et al., 2020). At least three individual complexes are observed en route to the final Type II complexes (Fig. 10A). The evidence indicates that the final complex is needed to achieve total inhibition of either 7-benzylquinolone O-dealkylation or testosterone 6β-hydroxylation. The inhibitory behavior is depicted in the traces in Fig. 10B, where the rate of 7-benzylquinoline O-debenzylation is not affected in the first 10 seconds after adding indinavir and then reaches an inhibited steady-state. The traces could be fit with a log-linear relationship, where the initial exponential phase involves first-order rearrangement of the initial P450 3A4-indinavir complex to the final E*I form (Fig. 10A).

One issue that was not addressed in our mechanistic work on P450 3A4 inhibition was whether both a substrate and inhibitor could present together in the “active site” (Fig. 10A), a question that arose earlier with cholesterol and nifedipine (and quinidine) (Fig. 5) (Shinkyo and Guengerich, 2011). Ketoconazole is a relatively large molecule (formula weight 531, 630 Å³), but an X-ray crystal structure of P450 3A4 showed occupancy by two ketoconazole molecules (Fig. 11) (Ekroos and Sjögren, 2006). To date, no P450 structures have been published with two different ligands present, although the possibility exists. Nevertheless, the size of the active site and the precedent with two ketoconazole molecules (Ekroos and Sjögren, 2006) indicate that this should be possible, making the kinetics even more complex.

Although P450 17A1 also showed similar sequences of spectral changes over a period of 10-30 seconds when binding inhibitors, inhibition proceeded very quickly (Child and Guengerich, 2020; Guengerich et al., 2021). The difference in behavior may be due to the large size of the active site (1400 Å³ (Yano et al., 2004)), compared to P450 17A1 (DeVore and Scott, 2012; Petrunak et al., 2014). Conclusions about one P450 do not necessarily apply to all others.

Fig. 9. Boundary line for $k_{\text{obs}}$ for time-dependent inhibition and relation to in vivo drug-drug interactions (DDI) (Eng et al., 2021). (A) Fifty drugs were evaluated for P450 3A4 time-dependent inhibition in human liver microsomes (at 30 µM unless noted otherwise) and ranked by $k_{\text{obs}}$, the first-order rate of inactivation, as judged using midazolam 1´-hydroxylation (O), presented on a log 10 scale (right y-axis). The filled bars show the in vivo drug-drug interactions as judged by the AUCR (AUC with the drug divided by the AUC without the drug. Clinical DDI magnitude). (B) The study in Part A was repeated in human hepatocytes. The stippled line indicates a 2-fold in vivo difference. Also indicated are p<0.05 statistical limits and a $k_{\text{obs}}$ “boundary” of the lowest in vitro value with 2-fold in vivo difference. Reprinted from Drug Metab. Dispos., Vol. 49, Eng, H., Tseng, E., Cerny, M. A., Goosen, T. C. and Obach, R. S., Cytochrome P450 P450 3A time-dependent inhibition assays are too sensitive for identification of drugs causing clinically significant drug-drug interactions: a comparison of human liver microsomes and hepatocytes and definition of boundaries for inactivation rate constants, pages 442-450 (Eng et al., 2021), Copyright (2021), with permission from American Society for Pharmacology and Experimental Therapeutics.
**EXAMPLES OF ISSUES WITH P450 INHIBITION**

Terfenadine is a rather classic example of a drug-drug interaction problem. Seldane®, containing terfenadine as the active ingredient, was the first non-sedating antihistamine on the market and by 1990 had been used by ~100 million people worldwide (Thompson and Oster, 1996; Guengerich, 2014). In 1989 an arrhythmia was observed in an individual who took an intentional overdose and by 1990 this “torsade des pointes” was also observed in some individuals using the recommended dose (Woosley et al., 1993; Woosley, 1996). The problem was identified as an accumulation of terfenadine in the plasma of those affected (Honig et al., 1992), exacerbated by erythromycin. Ultimately at least 140 deaths were attributed to terfenadine (Rango, 1997).

Our own laboratory demonstrated the involvement of P450 3A4 in the metabolism of terfenadine (Fig. 12) (Yun et al., 1993). This assignment was unknown at the time terfenadine was marketed, demonstrating how far the P450 science and the regulatory expectations have advanced since then. Terfenadine has high affinity for the hERG potassium channel protein, which explains the undesired pharmacological effect.

The role of P450 3A4 explains the interactions with erythromycin, ketoconazole, and other drugs that led the U.S. FDA to first add a “Black Box” warning in 1992 and to eventually recall the drug in 1997. Other antihistamines without the hERG issue were developed, including loratadine.

Today new chemical entities are screened to establish roles of individual enzymes, particularly P450s, in metabolism and to predict what drug-drug interactions might occur. In addition, routine hERG screening is now done in many pharmaceutical companies.

Fexofenadine, the final oxidation product, is not a hERG ligand and has almost as much affinity for the H1 receptor as terfenadine. Being devoid of the negative aspects (and even having a more favorable cLogP value), it was developed (as Allegra®) and is still marketed today (Guengerich, 2014).

### GESTODENE

Gestodene is a “third-generation” progestin used in oral contraceptives (Fig. 13). It was discovered in 1975 and is used in several countries but was never approved in the United States. It is one of the lowest dose progestins, apparently because it is a very potent agonist of the progesterone receptor. Oral contraceptives also include EE2 as the estrogenic component.
EE₂ (Guengerich, 1988) and several other 17-acetylenic steroids (Ortiz de Montellano et al., 1979; Guengerich, 1990a) are mechanism-based inactivators of P450s, including P450 3A4, the enzyme involved in the 2-hydroxylation of EE₂ (Guengerich, 1988).

Of a series of acetylenic contraceptive steroids tested, gestodene was the most potent in terms of inactivating P450 3A4 (Guengerich, 1990a). The inactivation was highly selective for P450 3A4 (Guengerich, 1990a). The presence of the 15,16-double bond is important, in that the rate of inactivation of P450 3A4 by gestodene is 5-fold faster than levonorgestrel (Fig. 13). In in vitro experiments, gestodene has a $k_{inactivation}/K_a$ ratio ≥10-fold higher than EE₂; in inhibiting P450 3A4 irreversibly (Guengerich, 1990a). In vivo, formulations containing both gestodene and EE₂ lead to an increased $C_{p,max}$ and AUC of EE₂ with time (~50% over 21 days) (Kuhl et al., 1988). The major pathways of metabolism of gestodene itself involve reduction of the 3-keto group and C1, C6, and C11 hydroxylation (Kuhl et al., 1995), probably by P450 3A4 (Ward and Back, 1993). During repeated use, the pharmacokinetics of gestodene also change. Both of these changes may be related to the inhibition of P450 3A4 by gestodene (Kuhl et al., 1988; Guengerich, 1990b; Kuhl et al., 1995). However, the amount of P450 3A4 in a human liver far exceeds the amount of gestodene used each day, as noted earlier (Guengerich, 1990a). It has also been shown that the in vivo clearance of midazolam was not considered modified by administration of gestodene (Palovaara et al., 2000). However, in vivo midazolam oxidation (following oral administration) is regarded to be indicative of hepatic P450 3A4. One explanation is that the metabolism of EE₂ and gestodene is primarily intestinal and that gestodene inactivates that pool of P450 3A4 but not that in liver, as seen with grapefruit juice and bergamottin (Paine et al., 2004). It is of interest that women taking oral contraceptives containing gestodene are 5-6 times more likely to develop venous thromboembolism than women not using contraceptive pills and 1.6 times as likely compared to those taking contraceptives containing levonorgestrel (Fig. 13) (Lidegaard et al., 2011). How this might be related to P450 3A4 inactivation is not clear.

**BERGAMOTTIN**

In 1990 a classical clinical drug interaction study led to an unexpected finding. An ethanol interaction study was done with the anti-hypertensive drug felodipine, a dihydropyridine calcium channel blocker. In these studies, fruit juice is often used to mask any taste of alcohol in order to prevent subjects from knowing what they were consuming. There was no effect of ethanol but grapefruit juice itself led to a dramatic increase in the AUC for orally administered felodipine (Edgar et al., 1990; Bailey et al., 1991). Subsequent studies showed similar AUC increases for several other orally administered P450 3A4 substrates (Bailey et al., 1993) (one of which is felodipine (Guengerich et al., 1991)). This effect was not seen with orange juice but could later be demonstrated with Seville orange juice and starfruit juice (Malhotra et al., 2001).

The search for grapefruit-specific natural products led to examination of naringenin but this was a weak inhibitor (Guengerich and Kim, 1990). Ultimately the furanocoumarin bergamottin was implicated (He et al., 1998). The mechanism is now known in detail, including the site of P450 3A4 that is modified (Fig. 14).

The phenomenon is now well-known, and many P450 3A4 substrates have warnings in their labels. Although this phenomenon has now been recognized for 30 years, apparently there have been no reported fatalities. The amount of bergamottin in a large serving of grapefruit juice (or grapefruit itself) is enough to produce a sizeable effect on AUC, but the phe-
nomenon appears to be largely restricted to drugs that show extensive first-pass intestinal clearance (Schmiedlin-Ren et al., 1997). P450 3A4 is the major P450 in the human small intestine (Paine et al., 2005) but the amount of it there is only a few percent of that in the liver (Guengerich, 1990a). Presumably even if a large fraction of the P450 3A4 in the small intestine is inactivated, the hepatic P450 3A4 can oxidize the fraction that enters the liver through portal circulation.

The discovery of the inhibitory effect of a natural product in food was rather serendipitous (Dresser et al., 2000) but there are probably similar compounds in foods that have yet to be discovered (Goosen et al., 2004). Herbal medicines and dietary supplements are not innocuous, e.g. St. John’s wort contains the powerful PXR inducer hyperforin (Moore et al., 2000) and searches are in order for other inhibitors in natural products (Paine et al., 2018).

**PRACTICAL ISSUES IN DEALING WITH P450 INHIBITION**

As molecules are discovered with biological activity in a pharmaceutical program, early screening for P450 inhibition is often done to help stratify the compounds for further consideration. With the current knowledge of marker activities, it is possible to do screening rapidly. A sequential approach such as that shown in Table 4 is often used, although assays for time-dependent inhibition may often precede determination of $K_i$ values (the design for $K_i$ determination will differ in a time-dependent reaction). These screens are done in *in vitro* with either recombinant human enzymes or with cells or extracts of human tissues.

How does one deal with the results of such studies, and how much inhibition is a problem? A simplified approach is outlined in Fig. 15 (Obach et al., 2005; Shou and Dai, 2008). The predicted change in the exposure (AUC) for a drug is a function of the concentration of the inhibitor (I) and its $K_i$ value. If multiple P450s (CYPs) are involved in the disposition of a “victim” drug, the fraction of the metabolism attribute to each P450 is $f_{m(CYP)}$. The *in vitro* $K_i$ value can be applied along with the inhibitor concentration. What is important is the unbound plasma concentration of the inhibitory drug. This analysis may seem straightforward, but one of the major issues is predicting what the plasma concentration of a new drug will be when used in patients. Obviously it is desirable to develop drugs with high efficacy in order to keep dosages lower and avoid drug-drug interactions.

A practical flow chart that came out of an FDA draft is presented in Fig. 16. The right side of Fig. 16 deals with issues of drug inhibition. In some cases dose adjustment may be in order, but that can result in a loss of drug efficacy. Another flow chart from the same FDA Draft Guidance is shown in Fig. 17, which includes mention of a “sensitive” probe substrate. Some drugs are more sensitive to interference from inhibitors than others, and in turn some of these have narrow therapeutic windows (Table 5). That is, there are potentially dangerous consequences of having a concentration of the drug either to low or too high. A classic example is warfarin. Too low a level of this anti-coagulant leads to risk of stroke but too high a level can cause dangerous hemorrhaging.

The FDA has classified inhibitors on the basis of AUCR, the ratio of AUC without inhibitor compared to AUC with the in-
**Fig. 15.** Formulae used to estimate *in vivo* inhibition parameters from *in vitro* measurements (Obach *et al*., 2005). AUC: area under the curve; I, inhibitor (*in vitro* or plasma concentration); CL: clearance; \( f_{m(CYP)} \), fraction of the clearance of the drug catalyzed by a particular P450 enzyme (CYP).

\[
\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{1 + \frac{[I]_{in vitro}}{K_i}} \quad \text{(i.e., 50% inhibition when } [I] = K_i) \\
\frac{CL_{control}}{CL_{inhibited}} = \frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{1 + \frac{[I]_{in vitro}}{K_i}} \left[ \frac{f_{m(CYP)}}{1 + \frac{[I]_{in vitro}}{K_i}} + [1 - f_{m(CYP)}] \right]
\]

In principle it follows that:

\[
\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{1 + \frac{[I]_{plasma}}{K_i}}
\]

**Fig. 16.** An FDA decision tree scheme for metabolism-based drug-drug interaction studies (FDA, 2012).

**DRUGS DESIGNED TO INHIBIT P450S**

Most of the discussion in this review has been about avoid-
Inhibition of P450 Enzymes by Drugs

Guengerich.    Inhibition of P450 Enzymes by Drugs

Figure 17. An FDA general scheme of model-based prediction. In this scheme (FDA, 2012), the investigational drug (and any metabolite present at ≥25% of the parent drug AUC) is considered as an interacting drug with P450 enzymes. TDI: time-dependent inhibition; DDI: drug-drug interaction; AUC<sub>R</sub> (AUC<sub>ratio</sub>): AUC<sub>with drug</sub> / AUC<sub>without drug</sub> ; R=1+([I]/K<sub>i</sub>). For R<sub>alt</sub> (for oral dosage of P450 3A4 inhibitors), I=I<sub>gut</sub>=molar dose/250 mL. For the calculation of AUC<sub>R</sub>, A, B, and C denote terms for time-dependent inhibition, induction, and reversible inhibition, respectively, in the gut (subscript g) or liver (subscript h). F<sub>g</sub> is the fraction of the drug escaping first-pass intestinal metabolism, and f<sub>m</sub> is the fractional contribution of a particular P450 (e.g., 3A4) to the metabolism of the drug in the liver (Fahmi et al., 2009). A is a function of the rate of degradation of the P450 (k<sub>deg</sub>) and the rate constant for the time-dependent inhibition (k<sub>inactivation</sub>), B is a function of parameters associated with induction of the particular P450, and C is a simple ratio of the free inhibitor concentration and K<sub>i</sub> (Fig. 15) (Fahmi et al., 2009).
levels of the 17α-hydroxy steroids that are needed to produce cortisol and aldosterone, precluding patients to hyperkalemia and hypertension, which can only be partially alleviated by supplemental prednisone (Mostaghel and Nelson, 2008; Attard et al., 2012). An ideal drug would inhibit only the lyase step and not the hydroxylation, but this may not be feasible with an active site that must accommodate both a substrate and an inhibitor.

A number of drugs, including mifepristone (Chu et al., 2001), have been used to inhibit adrenal P450 11B1 in treating Cushing’s disease, which is a syndrome involving overproduction of cortisol (Boscaro et al., 2000; Yin et al., 2012; Emmerich et al., 2013, 2017).

P450 51 enzymes are involved in 14α-demethylation of ste-
rols. In mammals, P450 51A1 is a lanosterol 14α-demethylase, catalyzing a key step in the synthesis of cholesterol. This enzyme has been considered as a target for cancer treatment (Friggeri et al., 2019), but in general human P450 51A1 is not considered a drug target (statins are more effective drugs, targeting HMG CoA-reductase). Fungi and yeasts also have P450 Family 51 enzymes, needed for the production of ergosterol for membrane synthesis. This has proven to be a major target for anti-fungals (Table 6), which are used to treat simple problems (e.g., athlete’s foot—tinea pedis) as well as major target for anti-fungals (Table 6), which are used to treat life-threatening systemic fungal infections common in immunocompromised individuals (Chen et al., 2020). P450 51A1 (breast and other hormonal cancers) Exemestane Anastrozole Letrozole P450 17A1 (prostate cancer) Abiraterone P450 11B1 (Cushing’s disease) Mifepristone P450 51 (anti-fungal, inhibit fungal P450s) Ketoconazole Fluconazole Itraconazole Vorconazole Posaconazole Isavuconazole Mifepristone

| Discovery and development programs | |
|------------------------------------|-----------------|
| P450 4A11 (hypertension)           |                 |
| P450 11A1 (prostate cancer)        |                 |
| P450 11B2 (hypertension)           |                 |
| P450 2A41 (increase vitamin D, levels) |           |
| P450 2A61 (increase vitamin A levels) |             |
| P450 2B61 (increase vitamin A levels) |             |

P450 2A6, for instance, has been considered as a target because of its role in the metabolism of nicotine, with the idea that inhibition of nicotine metabolism would cause smokers to use fewer cigarettes (Sellers et al., 2000; Yano et al., 2006).

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