

# The cytochrome P450 isoenzyme and some new opportunities for the prediction of negative drug interaction in vivo

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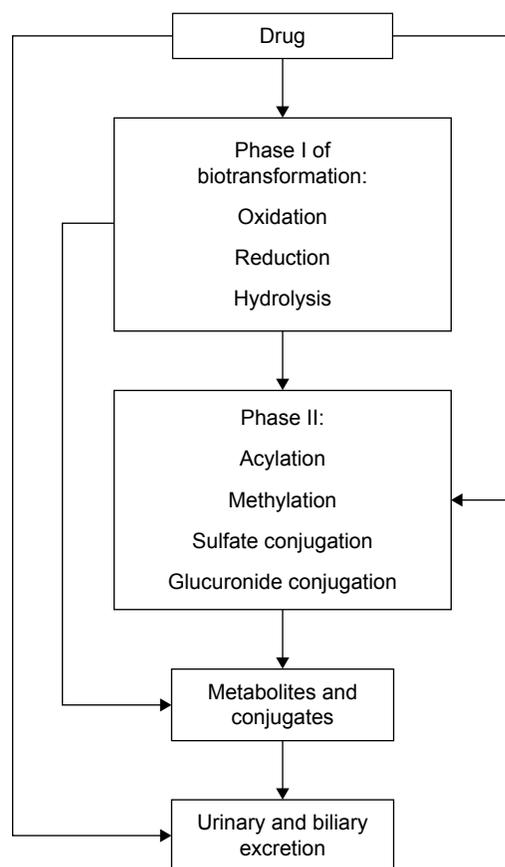
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**Abstract:** Cytochrome (CYP) 450 isoenzymes are the basic enzymes involved in Phase I biotransformation. The most important role in biotransformation belongs to CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2. Inhibition and induction of CYP isoenzymes caused by drugs are important and clinically relevant pharmacokinetic mechanisms of drug interaction. Investigation of the activity of CYP isoenzymes by using phenotyping methods (such as the determination of the concentration of specific substrates and metabolites in biological fluids) during drug administration provides the prediction of negative side effects caused by drug interaction. In clinical practice, the process of phenotyping of CYP isoenzymes and some endogenous substrates in the ratio of cortisol to 6 $\beta$ -hydroxycortisol in urine for the evaluation of CYP3A4 activity has been deemed to be a quite promising, safe and minimally invasive method for patients nowadays.

**Keywords:** cytochrome CYP450, drug interaction, drug metabolism, phenotyping

## Introduction

It is well known that efficacy and safety of drugs used in clinical practice mainly depend on drug concentration near target molecules. This concentration usually correlates with plasma drug concentration, which depends on absorption, distribution and elimination processes. Elimination includes biotransformation and/or excretion of a drug (Figure 1). The main enzymes of oxidation reaction in Phase I are cytochrome (CYP) 450 isoenzymes, alcohol dehydrogenase, aldehyde dehydrogenase and aminooxidase.<sup>1</sup> Recently, a number of studies devoted to endogenous and exogenous markers of CYP expression and activity were published. The results of those investigations could be promising in the clinical practice for the prediction of the patient's personal reaction to medication and negative drug interactions. Diczfalusy et al<sup>2</sup> reported that 4 $\beta$ -hydroxycholesterol (4 $\beta$ -OHC), which is formed by CYP3A4 from cholesterol, is a promising endogenous marker for the prediction of negative drug interactions with inducers of CYP3A enzymes or CYP3A inhibitors such as ritonavir or itraconazole. In this review, the authors also established a relationship between the concentration of 4 $\beta$ -OHC and the number of active CYP3A5\*1 alleles showing that 4 $\beta$ -OHC was formed not only by CYP3A4 but also by CYP3A5 and thus proved that the above-mentioned correlations depend on gender as well (women had higher concentration of 4 $\beta$ -OHC in comparison with men). 4 $\beta$ -OHC has half-life of 17 days, and this is the reason why, in short-term studies, exogenous markers such as midazolam or quinine probes may be superior; but in long-term studies, 4 $\beta$ -OHC is a sensitive marker of



**Figure 1** Phases of biotransformation.

CYP3A activity especially to assess drug interactions caused by induction and inhibition of CYP.<sup>2</sup> CYP3A4 converts cortisol CYP3A4 to 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHC), which is used as an endogenous marker in urine for the determination of CYP3A activity. CYP3A4 also converts cholesterol to another marker 4 $\beta$ -OHC.<sup>2</sup> Mårde Arrhén et al<sup>3</sup> compared the ratio of 4 $\beta$ -OHC to cholesterol in plasma with the ratio of 6 $\beta$ -OHC to cortisol in urine after the administration of rifampicin for 2 weeks, and after the analysis of obtained data, they concluded that the abovementioned markers give similar results about CYP activity. The concentration of cortisol and its metabolites in the body is flexible and depends on chronobiological rhythms; that is why the use of the ratio of 6 $\beta$ -OHC to cortisol is more rational for the precision prediction of CYP3A4 induction by rifampicin.<sup>3</sup> In their comparison of endogenous 4 $\beta$ -OHC with midazolam as markers for CYP3A4 induction by rifampicin, Björkhem-Bergman et al<sup>4</sup> showed that the 4 $\beta$ -OHC ratio is comparable with midazolam clearance as a marker of CYP3A4 induction, and each may be used to evaluate CYP3A4 induction in clinical trials evaluating drug–drug interactions for new drugs. These data are in good agreement with the data of Kasichayanula et al,<sup>5</sup>

which showed that changes in the plasma of 4 $\beta$ -OHC can be utilized as a surrogate for midazolam pharmacokinetics after the administration of multiple doses of potent CYP3A inducers (rifampicin). There is a more limited dynamic range for 4 $\beta$ HHC for the assessment of potential CYP3A inhibitors. This review also confirmed the opinion of previous authors that 4 $\beta$ -OHC is a valuable marker for the assessment of potential CYP3A inducers in early drug development and particularly in drug interaction.<sup>5,6</sup> This review is devoted to the recent data about CYP isoenzyme and some new opportunities for the prediction of negative drug interaction in vivo.

## Role of CYP isoenzymes in drug metabolism

The major biotransformation enzyme is CYP, which has more than 1,000 isoenzymes, of which five (CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2) metabolize 90% of all drugs (Table 1).<sup>7</sup> CYP is a group of enzymes first isolated from liver microsomes of rats separately by Klingenberg<sup>8</sup> and Garfinkel.<sup>9</sup> Nomenclature of CYP includes family (CYP2, CYP3, etc.), subfamily (CYP2D, CYP3A, etc.) and the name of definite isoenzyme (CYP2D6, CYP3A4, etc.).<sup>10</sup> CYP can be found in hepatocytes as well as in other cells of the body. According to the analysis of 200 most commonly prescribed drugs in the US (2002), about 73% of drugs are subject to metabolism, of which about 75% of the drugs are metabolized by CYP.<sup>11</sup> Type CYP3A isoenzyme metabolizes 46% of drugs, type CYP2C9 isoenzyme metabolizes 16% of drugs, type CYP2C19 + CYP2D6 isozymes metabolize 12% of drugs, type CYP1A isoenzyme metabolizes 9% of drugs and type CYP2B6 + CYP2E1 isoenzymes metabolize 2% of drugs.<sup>12</sup> Substrate specificity is an inherent characteristic of CYP isozymes, ie, they have the ability to bind and transform molecules with specific shape, charge and hydrophilic/hydrophobic characteristics.<sup>13,14</sup> Some of the CYP isozymes have substrate stereospecificity, for example, CYP2C9 metabolizes S-warfarin (more active enantiomer of warfarin) and R-warfarin is metabolized by CYP1A2 and CYP3A4.<sup>15,16</sup>

**Table 1** The relative content of CYP isoenzymes in the liver, their basic share and their participation in the metabolism of drugs

CYP isoenzyme	Content in the liver (%)	Contribution to metabolism (% metabolizing drugs)
1A2	~13	8.2
2C	~18	15.8 (2C8, 2C9)
2D6	Up to 2.5	18.8 (2C18, 2C19)
3A4	Up to 28	34.1

**Note:** Data from Kasichayanula et al.<sup>5</sup>

**Abbreviation:** CYP, cytochrome.

**Table 2** Typical substrates of basic CYP isozymes

CYP isozyme	Substrates
CYP1A2	Clozapine, caffeine, paracetamol, theophylline, phenacetin, R-warfarin
CYP2C9	Hexobarbital, zidovudine, losartan, paracetamol, testosterone, tolbutamide, phenytoin, celecoxib, S-warfarin
CYP2C19	Hexobarbital, diazepam, zidovudine, omeprazole, pantoprazole, testosterone, phenytoin, R-warfarin, S-warfarin
CYP2D6	Haloperidol, dextromethorphan, codeine, metoprolol, nortriptyline, paracetamol, pravastatin, propafenone
CYP3A4	Alprazolam, atorvastatin, vincristine, halothane, hydrocortisone, zidovudine, carbamazepine, codeine, cortisol, caffeine, lidocaine, lovastatin, midazolam, nifedipine, paracetamol, tacrolimus, tamoxifen, testosterone, phenytoin, cyclosporine, cyclophosphamide, erythromycin, R-warfarin, S-warfarin

**Note:** Data from Wadelius et al.<sup>57</sup> and Kasichayanula et al.<sup>5</sup>

**Abbreviation:** CYP, cytochrome.

Most isoenzymes demonstrate broad substrate specificity, ie, each isozyme is able to metabolize a wide spectrum of xenobiotics, including drugs<sup>1</sup> (Table 2). The activity of CYP isoenzymes may vary over a wide range after the exposure of inducers or inhibitors, thereby altering the metabolism of substrates of isoenzymes, which could result in drug–drug interactions.<sup>17</sup> Isoenzyme CYP3A4 metabolizes approximately 40%–50% of the drugs used in clinical practice, including calcium slow channel blockers,<sup>18</sup> macrolide antibiotics,<sup>19</sup> statins (simvastatin, lovastatin, atorvastatin)<sup>20</sup> and some of the “new” oral anticoagulants from the group of direct factor Xa inhibitors (rivaroxaban, apixaban).<sup>21</sup> The most important inducers of CYP3A4 are carbamazepine,<sup>22</sup> phenobarbital,<sup>23</sup> phenytoin,<sup>24</sup> rifampicin<sup>25</sup> and St John’s wort extract.<sup>26</sup> Some antifungal agents from the azole group (ketoconazole, itraconazole),<sup>27</sup> protease inhibitors (indinavir, nelfinavir, ritonavir)<sup>28</sup> and clarithromycin<sup>29</sup> are the most important CYP3A4 inhibitors.

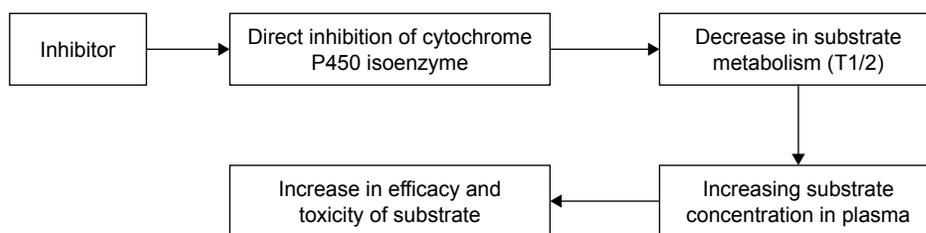
CYP2C9 is the main enzyme involved in the metabolism of many nonsteroidal anti-inflammatory drugs (NSAIDs): celecoxib,<sup>30</sup> diclofenac,<sup>31</sup> ibuprofen,<sup>32</sup> lornoxicam,<sup>33</sup> meloxicam,<sup>34</sup> naproxen,<sup>35</sup> S-warfarin,<sup>22</sup> many antidiabetic sulfonylureas (glibenclamide,<sup>36</sup> glimepiride,<sup>37</sup> glipizide,<sup>38</sup> tolbutamide<sup>39</sup>), angiotensin II receptor blockers (irbesartan,<sup>40</sup> losartan<sup>41</sup>), fluvastatin,<sup>42</sup> phenytoin,<sup>38,43</sup> tamoxifen<sup>44</sup> and cyclophosphamide.<sup>45,46</sup> The most important inducer of CYP2C9 is rifampicin.<sup>47</sup> Fluconazole and amiodarone are significant inhibitors of CYP2C9. CYP2C19 is the main metabolic enzyme for proton pump inhibitors, which in turn are the inhibitors of this isoenzyme (the so-called autoinhibitors).<sup>48,49</sup>

This enzyme is induced by carbamazepine, prednisolone and rifampicin.<sup>50,51</sup> CYP2D6 metabolizes up to 20% of drugs, including tricyclic antidepressant amitriptyline, neuroleptics and  $\beta$ -adrenoblockers. Codeine is metabolized by CYP2D6 into the active metabolite of morphine.<sup>52</sup> CYP2D6 is inhibited by fluoxetine,<sup>53</sup> quinidine<sup>54</sup> and bupropion.<sup>55</sup> Unlike other isozymes, CYP2D6 has no verified inducers,<sup>56</sup> but isoenzyme activity increases in the gestation period.<sup>57</sup> However, there are controversial data about its weak induction by dexamethasone and rifampicin.<sup>58</sup> CYP1A2 has no endogenous substrates. It metabolizes mainly xenobiotics, including theophylline, caffeine and acetaminophen. Autoinducers of CYP1A2 are polycyclic aromatic hydrocarbons (the main component of inhaled tobacco smoke), which by means of CYP1A2 are transformed into carcinogenic compounds.<sup>59</sup> Besides, components of tobacco, smoke, grilled meal<sup>60</sup> and broccoli<sup>61</sup> are inducers of CYP1A2. Ciprofloxacin and fluvoxamine inhibit CYP1A2.<sup>62</sup>

Gene polymorphism in many CYP isozymes may be responsible for the interindividual differences in the speed of biotransformation of drugs and certain drug–drug interactions.<sup>63</sup> The presence of single-nucleotide polymorphism (SNP) in the gene encoding a particular isozyme may lead to the synthesis of enzymes with altered activity, which in turn changes the pharmacokinetics of drugs metabolized by this isoenzyme. In clinical practice, it is possible to conduct pharmacogenetic testing of some drugs to identify genetic polymorphisms.<sup>42</sup> This allows to predict the pharmacological response to these drugs, which in turn increases the efficacy and safety of drug therapy.<sup>50</sup> CYP isoenzymes play a pivotal role in the metabolism of many drugs used in clinical practice. The changes in isoenzymes activity are the basis of drug–drug interaction at the level of biotransformation; thus, it is important to study the effect of drugs on CYP isoenzymes for safe pharmacotherapy.<sup>50</sup>

## Inhibition of CYP isoenzymes

The inhibition of CYP isoenzymes is a particular mechanism of clinically significant drug interaction (Figure 2). A decrease in the metabolism of drugs which are substrates for the abovementioned enzyme leads to increased concentration of these drugs in plasma and exerts toxic effects.<sup>50,64</sup> Inhibition of isoenzymes could be reversible as well as irreversible. The reversible mechanisms of inhibition can be competitive, noncompetitive and uncompetitive. In the type of competitive reversible inhibition, the drug inhibitor and drug substrate compete for the active site of isoenzyme; therefore, this type of inhibition could be overcome by increasing the concentration of drug substrate.<sup>65</sup> Mechanism of noncompetitive reversible



**Figure 2** Schematic of drug–drug interaction between substrate and inhibitor of CYP isoenzyme.

**Note:** Data from Ritter et al.<sup>72</sup>

**Abbreviation:** CYP, cytochrome.

inhibition is due to the binding of inhibitor with the nonfunctional part of CYP isoenzyme, which changes the conformation of active site and prevents its binding to the drug substrate; therefore, this type of inhibition cannot be reversed by increasing the concentration of drug substrate. Noncompetitive inhibition of CYP isoenzymes is observed upon binding of the inhibitor to isoenzyme–substrate complex.<sup>66</sup>

There are two types of irreversible inhibition: true irreversible inhibition and quasi-irreversible inhibition. Under true irreversible inhibition, the inhibitor or its intermediate metabolite covalently binds to the heme of CYP isoenzyme, thereby inactivating it. A strong noncovalent bond is formed between inhibitor and CYP isoenzyme in the case of quasi-irreversible inhibition. During irreversible inhibition, the recovery time of isoenzyme activity depends on the time needed for the synthesis of a new isoenzyme. Inhibitors of CYP isoenzymes (Table 3) may be classified in vivo

according to the degree of inhibitory activity of drug substrates of the isoenzymes. The use of potent inhibitors result in more than five times increase in area under the curve (AUC) of drug substrate (a decrease in clearance of >80%); moderate inhibitors increase the AUC of drug substrate by two to five times (a decrease in clearance up to 50%–80%) and weak inhibitors are able to increase the AUC of drug substrate by 1.25–2 times (a decrease in clearance up to 20%–50%).<sup>67</sup>

### Induction of CYP isoenzymes

The absolute increase in the number and/or catalytic activity of isoenzymes and the associated reduction in the concentration of drug, which are substrate for CYP isoenzymes, are a result of the induction of isoenzymes. In most cases, the induction of isozymes is clinically expressed with the decrease in the pharmacological effects. In some cases, the induction of CYP isoenzymes results in the increase

**Table 3** Inhibitors of major CYP isozymes

CYP isozyme	Potent inhibitors	Moderate inhibitors	Weak inhibitors
1A2	Ciprofloxacin, enoxacin, fluvoxamine	Zileuton, mexiletine, methoxsalen, felbamate, thiabendazole, phenylpropranolamine	Allopurinol, acyclovir, verapamil, disulfiram, caffeine, norfloxacin, propafenone, propranolol, ticlopidine, famotidine, cimetidine, echinacea extract
2C9		Amiodarone, miconazole, oxandrolone, fluconazole	Voriconazole, zafirlukast, capecitabine, co-trimoxazole (sulfamethoxazole + trimethoprim), metronidazole, sulfipyrazone, tigecycline, fluvastatin, fluvoxamine, etravirine
2C19	Fluconazole, fluvoxamine, ticlopidine	Fluoxetine, moclobemide, omeprazole, voriconazole	Armodafinil, carbamazepine, cimetidine, ethinyl estradiol, etravirine, somatotropin, felbamate, ketoconazole
2D6	Bupropion, paroxetine, fluoxetine, quinidine	Duloxetine, terbinafine, cinacalcet	Amiodarone, vemurafenib, verapamil, gefitinib, hydralazine, hydroxychloroquine, desvenlafaxine, diltiazem, diphenhydramine, imatinib, methadone, oral contraceptives, propafenone, ranitidine, ritonavir, sertraline, telithromycin, febuxostat, celecoxib, cimetidine, echinacea extract, escitalopram
3A4	Voriconazole, grapefruit juice (high concentration), itraconazole, ketoconazole, clarithromycin, lopinavir, nefazodone, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, conivaptan	Aprepitant, verapamil, grapefruit juice (normal concentration), darunavir, diltiazem, imatinib, ritonavir, fluconazole, fosamprenavir, ciprofloxacin, erythromycin	Alprazolam, amiodarone, amlodipine, atorvastatin, bicalutamide, zileuton, isoniazid, nilotinib, oral contraceptives, ranitidine, ranolazine, ticagrelor, tipranavir, fluvoxamine, fluoxetine, cyclosporin, cilostazol, cimetidine, an extract of goldenseal Canadian, Ginkgo biloba extract

**Note:** Data from Wu et al.<sup>52</sup>

**Abbreviation:** CYP, cytochrome.

in pharmacological effects (in the case of the formation of active metabolites) and even in toxic effects. For example, the induction of CYP2E1 resulted in increased metabolism of acetaminophen and increased formation of hepatotoxic metabolite *N*-acetyl-*p*-benzoquinone imine.<sup>68</sup>

The most important mechanism for the induction of CYP isozymes is the interaction of inducer with specific intracellular receptors that in fact are transcription regulator proteins (pregnane-X-receptor, androstane constitutive receptor, aryl-hydrocarbonate receptor, etc.),<sup>69</sup> thus forming a receptor-inducer complex. This complex penetrates into the cell nucleus and acts on the regulatory region of the gene that results in increased expression of the gene encoding CYP. There are mechanisms of induction, which are not associated with exposure to specific receptors. For example, the induction of CYP2E1 is associated with posttranscriptional stabilization of molecules of this isoenzyme.<sup>70</sup> Based on the degree of induction of biotransformation of drug substrates in vivo, inducers are classified into three types: potent ( $\geq 80\%$  reduction in AUC), moderate (50%–80% reduction in AUC) and weak (20%–50% reduction in AUC).<sup>67</sup> Typical inductors are summarized in Table 4. Usually, it takes several days for the development of drug–drug interactions between drug inducer of CYP isoenzyme and drug substrate of this isoenzyme, because the mechanism of induction of CYP isoenzymes includes the induction of gene transcription encoding and subsequent synthesis.

## Legal status of studies on the effect of drugs on biotransformation enzymes

The most important step in the safety assessment of new and already registered drugs is a comprehensive evaluation of their potential side effects.<sup>71</sup> The demographic aging of the population and increase in the number of drugs used by patients (including nonprescription drugs) have enhanced the risk of drug–drug interactions.<sup>60</sup> Extensive study is being conducted worldwide to identify potential risk of negative

drug–drug interaction between new and registered drugs, and effective measures have been introduced to reduce the risk of side effects (dose adjustment, additional therapeutic monitoring, etc.).<sup>72</sup> Usually, in vitro studies are conducted before in vivo studies. The aim of screening investigation in vitro is to determinate the usefulness of studies in vivo. The Food and Drug Administration (FDA) has recommended the inclusion of instructions for medical use of drugs with information on the possible inhibitory or inducing effect on the biotransformation enzymes in the section describing pharmacokinetics. In the European Union also, pharmacokinetic studies have been designed to assess the influence of new drug(s) on the effects of well-known drug(s).<sup>67</sup> The results of studies of side effects of new drug(s) could be used to predict their interaction with other drug(s) on the basis of the identified mechanisms of interaction.

## Prediction of impact of drugs on CYP isoenzymes in vivo

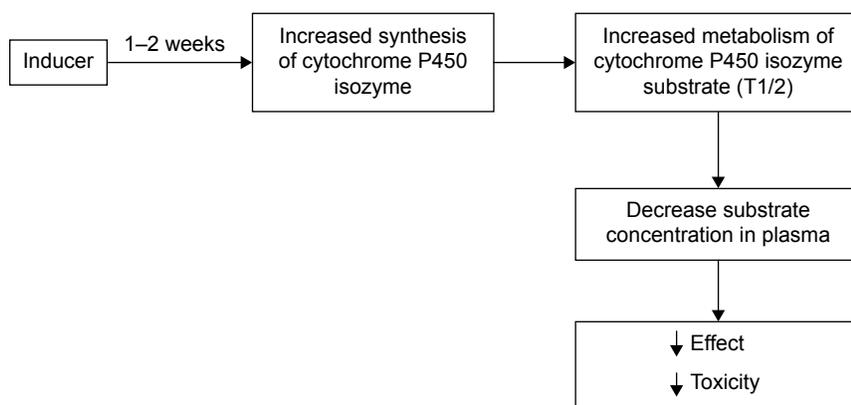
The study of potential side effects of new drugs includes conducting studies on the effects of new drugs on the pharmacokinetics of marker substrates of CYP isoenzymes, extrapolation of the results of such study to other drugs, substrates of isozymes and, in some cases, the study of specific combinations of drugs to be able to work out specific recommendations on combined use of drugs.<sup>60</sup> Marker substrates are substances that are metabolized primarily by one CYP isoenzyme (Figure 3). The purpose of the review for the investigation of influence of drugs on the marker substrates of CYP isoenzymes was to determine the presence or absence of any effect on CYP isoenzymes as well as to observe the magnitude of such influence, so as to classify the drugs that exhibit inductive/inhibitory properties in three groups: potent, moderate and weak. It is noted that Table 5 does not list all substrates that could be used to assess the effects of new drugs on the activity of CYP isoenzymes in vivo. For some CYP isozymes, methods for assessing the

**Table 4** Inductors of major CYP isozymes

CYP isozyme	Potent inducers	Moderate inducers	Weak inducers
1A2		Montelukast, tobacco smoke, phenytoin	Moricizine, omeprazole, phenobarbital
2C9		Arbamazepine, rifampicin	Aprepitant, bosentan, phenobarbital, St John's wort extract
2C19		Rifampicin, S-mephenytoin	Artemisinin
2D6	Inducers are not identified		Inducers are not identified
3A4	Avasimibe, carbamazepine, rifampicin, phenytoin, St John's wort extract	Bosentan, modafinil, nafcillin, etravirine, efavirenz, lurasidone	Aprepitant, armodafinil, pioglitazone, prednisolone, refinamid, echinacea extract

**Note:** Data from Wu et al.<sup>52</sup>

**Abbreviation:** CYP, cytochrome.



**Figure 3** Schematic of drug–drug interaction between the inducer and the substrate of CYP isoenzyme.

**Note:** Data from Ritter et al.<sup>72</sup>

**Abbreviation:** CYP, cytochrome.

activity were developed. The special feature of those methods is that they do not require the administration of any xenobiotics. For example, in one of the guidelines for pharmaceutical companies, it was recommended to study biotransformation and transporters of new drugs for the evaluation of CYP3A4 activity in vivo by using the ratio of endogenous cortisol to one of its metabolites, 6 $\beta$ -OHC, which is formed exclusively as a result of CYP3A4 activity<sup>73</sup> (Figure 4).

The past study showed that the dynamics of endogenous activity of CYP3A4 metabolic markers, including the ratio of 6 $\beta$ -OHC to cortisol in urine, correlate with midazolam clearance dynamics under the induction or inhibition of CYP3A4 in healthy volunteers; therefore, the ratio of 6 $\beta$ -OHC to cortisol in urine could be used to evaluate the activity of CYP3A4 in vivo.<sup>74</sup> Formation clearance of the sum of 6 $\beta$ -OHC and 6 $\beta$ -hydroxycortisone was shown to detect moderate and potent inhibition of CYP3A4 in vivo.<sup>75</sup> CYP2D6 activity may be evaluated using pinoline urinary metabolic ratio

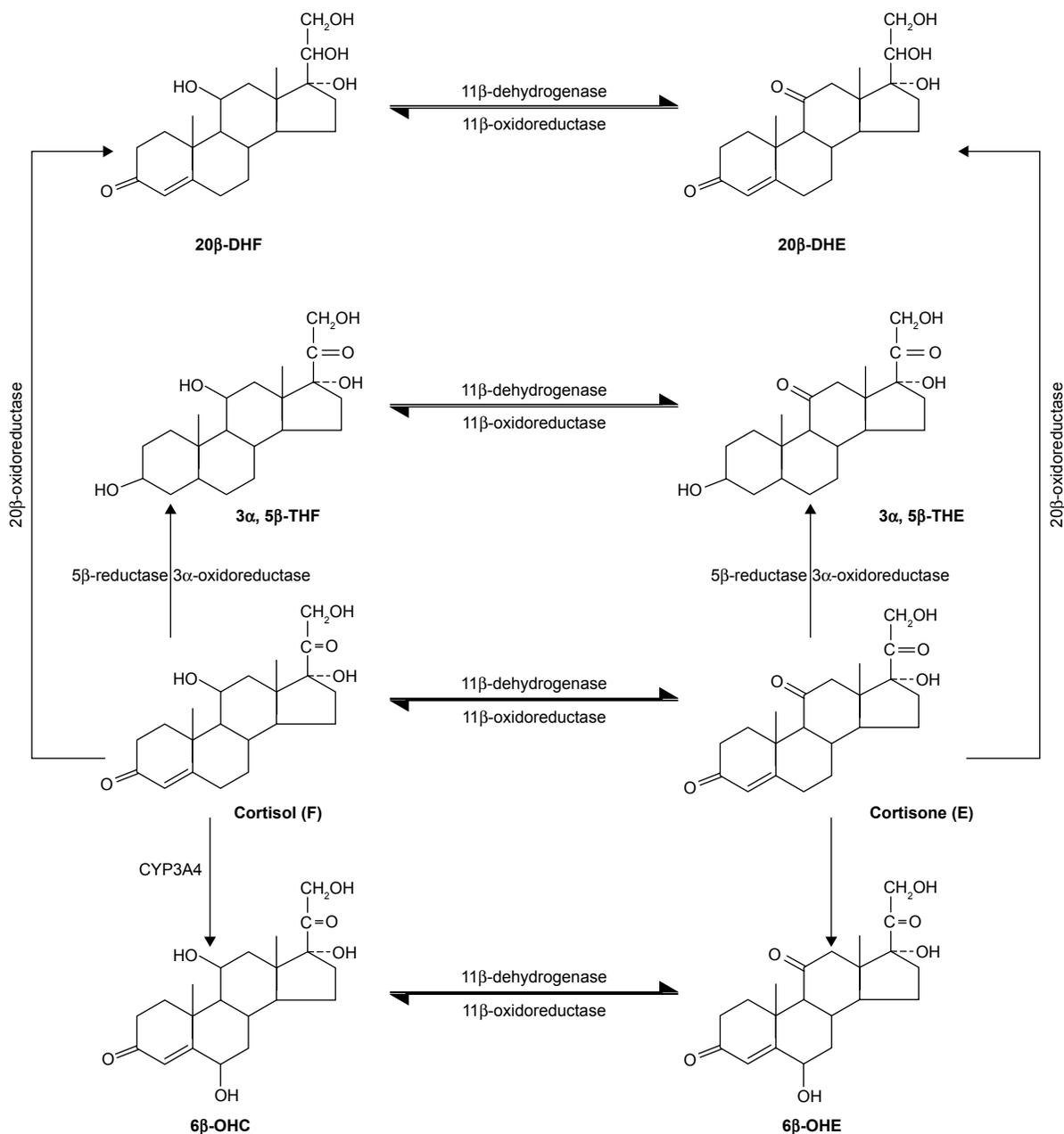
(6-methoxy-1,2,3,4-tetrahydro- $\beta$ -carboline/6-hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline).<sup>76</sup> To estimate CYP2C19 activity in vivo, omeprazole plasma hydroxylation index (omeprazole/5-hydroxyomeprazole) was introduced.<sup>77</sup> Losartan test could be used to assess the activity of CYP2C9. This test is based on the determination of concentration of losartan and its active metabolite, E-3174, which is produced mainly by CYP2C9 activity.<sup>78</sup> According to in vitro studies, E-3174 is also formed by the action of CYP3A4,<sup>79,80</sup> but in vivo studies with therapeutic doses of losartan showed no significant contribution to losartan CYP3A4 metabolism.<sup>81</sup> The predominant contribution of CYP2C9 to losartan metabolism was confirmed indirectly by a decrease in the AUC of E-3174 when it was used in combination with moderate inhibitor CYP2C9 (fluconazole), while no change in AUC of E-3174 was observed when losartan was used in combination with a strong CYP3A4 inhibitor (itraconazole).<sup>47</sup> In many studies, losartan has been safely used as a marker substrate for

**Table 5** Examples of sensitive substrates of basic CYP isoenzymes and substrates with a narrow therapeutic range

CYP isozyme	Sensitive substrate <sup>a</sup>	Substrates with a narrow therapeutic range
1A2	Alosetron, duloxetine, caffeine, melatonin, ramelteon, tacrine, tizanidine	Theophylline, tizanidine
2C9	Celecoxib	Warfarin, phenytoin
2C19	S-mephenytoin, clobazam, lansoprazole, omeprazole	S-mephenytoin
2D6	Atomoxetine, venlafaxine, desipramine, dextromethorphan, metoprolol, nebivolol, perphenazine, tolterodine	Pimozide, thioridazine
3A4	Alfentanil, aprepitant, budesonide, buspirone, vardenafil, dasatinib, darifenacin, darunavir, dronedarone, indinavir, quetiapine, conivaptan, lovastatin, lopinavir, lurasidone, maraviroc, midazolam, nisoldipine, saquinavir, sildenafil, simvastatin, sirolimus, ticagrelor, tipranavir, tolvaptan, triazolam, felodipine, fluticasone, everolimus, eletriptan, eplerenone	Alfentanil, astemizole, dihydroergotamine, pimozide, sirolimus, tacrolimus, terfenadine, fentanyl, quinidine, cisapride, cyclosporine, ergotamine

**Notes:** <sup>a</sup>The sensitive substrate is a substrate with AUC values more than fivefold increase in the combined use with an inhibitor of CYP isoenzymes. Data from Wadelius et al.<sup>57</sup>

**Abbreviations:** AUC, area under the curve; CYP, cytochrome.



**Figure 4** Metabolic conversion of cortisol.

**Note:** Data from Gray et al.<sup>85</sup>

**Abbreviations:** DHF, 20β-DHF (20β-dihydrocortisol); DHE, 20β-DHE(20β-dihydrocortisone); THF, 3α,5β-THF (3α,5β-tetrahydrocortisol); THE, 3α,5β-THE (3α,5β-tetrahydrocortisone); OHC, 6 β-OHC(6 β-hydroycortisol); OHE, 6 β-OHE(6 β-hydroycortisone).

phenotyping CYP2C9 activity.<sup>82-84</sup> Relative clinical safety of losartan and reliability of losartan test allow its usage in clinical studies dedicated to the investigations of the influence of new drugs on CYP2C9 activity in vivo.

## Conclusion and future perspectives

Prediction of negative drug interaction is one of the most important and difficult objectives of clinical pharmacology.

CYP isoenzymes are the basic enzymes involved in Phase I biotransformation. The use of CYP isoenzymes, cortisol to 6β-OHC ratio, losartan test and other tests used for the estimation of the activity of CYP so as to investigate the influence of new drugs can yield new and prospective findings related to the prediction of negative drug interaction, which in turn can provide new insights for researchers and clinicians.

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## Disclosure

The authors report no conflicts of interest in this work.

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