CHAPTER II

LITERATURE REVIEWS
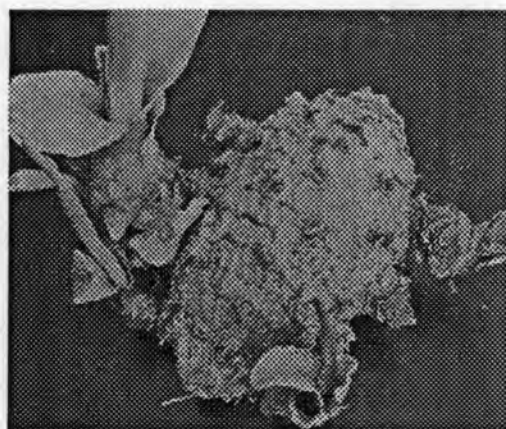
Curcuma comosa Roxb.

C. comosa Roxb. (Figure 1), a plant in family Zingiberaceae, has been traditionally used as an anti-inflammatory agent for the treatment of postpartum uterine inflammation. Natural compounds found in rhizomes of *C. comosa* can be classified on the basis of their chemical structures as following: (Suksamram et al., 1994, 1997)

1. diarylheptanoids: *trans*-1,7-diphenyl-5-hydroxy-1-heptene, *trans*-1,7-diphenyl-6-hepten-3-one-5-ol, *trans*-1,7-diphenyl-3-acetoxy-6-heptene, *trans*-1,7-diphenyl-6-heptene-3-one, *trans,trans*-1,7-diphenyl-1,3-heptadien-5-ol, *trans,trans*-1,7-diphenyl-4,6-heptadien-3-one, 1,7-diphenyl-1(*1E,3E,5E*)-heptatriene, 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(*1E*)-1-heptene),
   7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene

2. Acetophenones: 4,6-dihydroxy-2-*O*-(*β*-D-glucopyranosyl) acetophenone.

   The chemical structures of these following compounds are shown in Table 1.
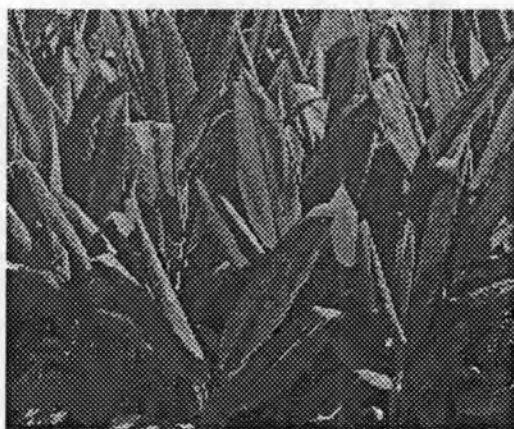
(A)   (B)

Figure 1 *Curcuma comosa* Roxb. plant (A) and rhizome (B)

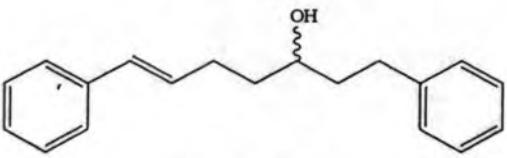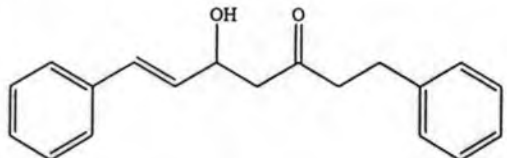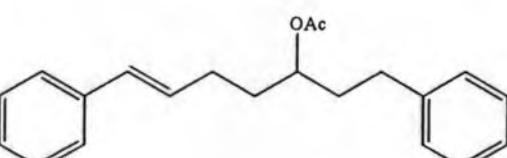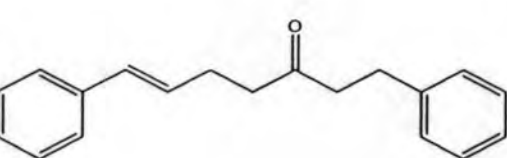**Table 1** Chemical structures of compounds found in rhizome of *C. comosa*
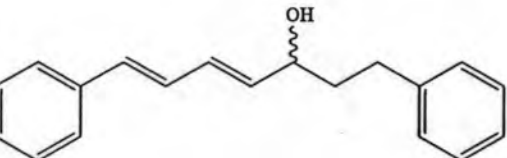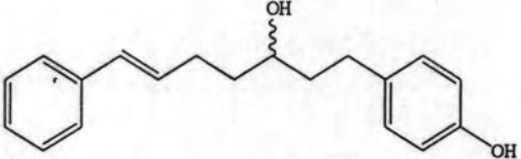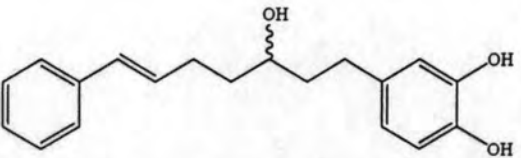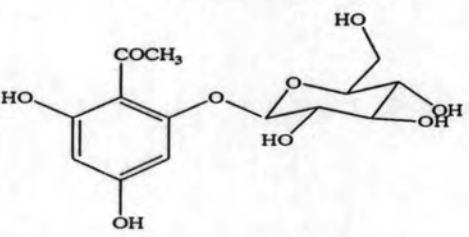
Compounds	Extraction fraction	Bioactivity	References
1. Diarylheptanoids *trans*-1,7-Diphenyl-5-hydroxy-1-heptene (1)	Hexane CH₃OH	Anti-inflammatory activity Nematocidal activity	Claeson et al., 1993 Jurgens et al., 1994
trans-1,7-Diphenyl-6-hepten-3-one-5-ol (2)	CH₃OH	Nematocidal activity	Jurgens et al., 1994
trans-1,7-Diphenyl-3-acetoxy-6-heptene (3)	CH₃OH	Nematocidal activity	Jurgens et al., 1994
trans-1,7-Diphenyl-6-heptene-3-one (4)	CH₃OH	Nematocidal activity	Jurgens et al., 1994
trans,*trans*-1,7-Diphenyl-1,3-heptadien-5-ol (5)	Hexane CH₃OH	Anti-inflammatory activity Nematocidal activity	Claeson et al., 1993 Jurgens et al., 1994

Table 1 (con't) Chemical structures of compounds found in rhizome of *C. comosa*

Compounds	Extraction fraction	Bioactivity	References
5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene (6)	EtOAc	Hypolipidemic activity Choleretic activity	Suksamrarn et al., 1994 Suksamrarn et al., 1997
7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene (7)	EtOAc	Choleretic activity	Suksamrarn et al., 1997
2. Phloracetophenone 4,6-dihydroxy-2-*O*-(*β*-D-glucopyranosyl) acetophenone (8)	EtOAc	Choleretic activity	Suksamrarn et al., 1997

Pharmacological effects

1. Uterotrophic effects

The hexane extract of *C. comosa* posseses a uterotrophic effect with the most effective in increasing uterine weight, glycogen content of bilaterally ovariectomized immature rats. It also induced cornification of vaginal epithelium, promoted growth

and induced keratinization of vaginal mucosa in ovariectomized mature rats (Piyachaturawat et al., 1995a).

Effects of *C. comosa* ethanolic extract on the contraction of intact and isolated rat uterus were investigated. The results showed that *C. comosa* ethanolic extract at the concentration of 5 and 10 μg/ml reduced uterine contraction, which induced by oxytocin, Ach, 5-HT and KCl in De Jalon solution. In addition, this extract at the concentration of 10 μg/ml reduced the contraction induced by oxytocin, vanadate and $PGF_{2\alpha}$ in Ca^{2+} free Locke Ringer solution with EGTA. In anesthetized rat, *C. comosa* ethanolic extract caused a decrease of uterine contraction induced by oxytocin (อนุกูล สวัสดิ์พาณิชย์, 2537).

2. Other estrogenic-like action

C. comosa hexane extract showed the suppression effects on male reproductive organs in immature rats. These effects were similar to the effects of estradiol (Piyachaturawat et al., 1998).

Effects of *C. comosa* hexane extract on male fertility in adult male rats were investigated. Intragastric administration of *C. comosa* hexane extract at a dose of 500 mg/kg/day for 7 consecutive days revealed effects similar to estradiol. Treatment with the hexane extract significantly decreased weights of testes, ventral prostate and seminal vesicles. Biochemical markers of accessory sex gland function such as prostatic acid phosphatase activity were decreased. The significant decrease in sperm concentration and motility were also observed in rat treated with the hexane extract at 500 mg/kg. However, this 7-day treatment did not significantly affect fertility of the animals (Piyachaturawat et al., 1999).

3. Choleretic effect

C. comosa revealed the choleretic effect. The butanol extract and ethyl acetate extract of *C. comosa* showed the greatest stimulatory effects on bile flow rate in rats. At high doses (1000 mg/kg) of these extracts, significant decrease bile salt concentration was observed while the total output of cholesterol, bilirubin and calcium were significantly increased (Piyachaturawat et al., 1996). Subsequently, a phloracetophenone glucoside (8) was isolated from the ethyl acetate extract and n-

butanol extract. It has been identified as the choleretic principle of the plant (Suksamrarn et al., 1996). Subsequent study in rat it was found that the aglucone of compound (8), phloracetophenone (2,4,6-trihydroxyacetophenone, THA) also showed choleretic activity. A single intraduodenal injection of THA significantly increased bile flow rate, bile acid concentration and output in a dose related manner whereas biliary cholesterol and phospholipid concentrations were decreased. In addition, THA significantly reduced plasma cholesterol (Piyachaturawat et al., 1996).

The study which explored the relationship between the chemical structure and choleretic activity of phloracetophenone (2,4,6-trihydroxyacetophenone) was undertaken. The study indicated that among 14 acetophenone analogues, 2,4,6-trihydroxyacetophenone possessed the most potent choleretic effect, which induced both high blood flow rate and a high bile salt output and led to lower plasma cholesterol levels in rats (Piyachaturawat et al., 2000).

In addition to the choleretic activity of phloracetophenone in rats, a study regarding effect of phloracetophenone in another species as hamsters was conducted. The study showed that hypercholesterolemic hamsters receiving THA 400 µmol/kg twice a day for 7 days had decreased plasma cholesterol and triglyceride level in a dose-related manner. The compound selectively reduced the elevated cholesterol in the VLDL and LDL fractions whereas cholesterol in HDL remained unchanged (Piyachaturawat et al., 2002).

4. Hypolipidemic effect

Simultaneously with the cholereletic activity, cholesterol lowering effect of C. comosa had been found in many studies. In hypercholesterolaemic hamsters, administration of C. comosa ethyl acetate extract at a dose of 500 mg/kg/day for 7 days caused a decrease of plasma cholesterol and triglyceride level (Piyachaturawat et al., 1999). Additionally, phloacetophenone (THA), the aglycone part of compound 8, also decreased plasma cholesterol in rats (Suksamran et al., 1997; Piyachaturawat et al., 1998) and decreased both plasma cholesterol and triglyceride level in hypercholesterolaemic hamsters (Piyachaturawat et al., 2002).

5. Nematocidal effect

Jurgens et al. (1994) reported that the methanolic extract of *C. comosa* was shown to have nematocidal activity against *Caenorhabditis elegans*. It was found that the most potent inhibitor of nematocidal mortality was *trans*-1,7-diphenyl-5-hydroxy-1-heptene (1), whereas *trans*-1,7-diphenyl-6-heptene-3-one (4) was slightly less active followed by *trans*-1,7-diphenyl-3-acetoxy-6-heptene (3) was less potent, and *trans* *,trans*-1,7-diphenyl-1,3-heptadien-5-ol (5) was inactive.

6. Anti-inflammatory effect

There was a study demonstrated an anti-inflammatory effect of *C. comosa*. The study showed that *C. comosa* hexane extract, ethanol extract and two diarrylheptanoids (5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1E)-1-heptene and 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1E)-1-heptene of *C.comosa* significantly decreased the release of pro-inflammatory cytokines, tumor necrosis factor α (TNF-α) and interleukin-1β, from phorbol-12-myristate-13-acetate (PMA)-stimulated PBMC and U937 cells. In PMA-stimulated U937 cells, both *C. comosa* diarylheptanoids reduced the expression of TNF-α, suppressed expression of IκB kinase and the activation of nuclear factor kappa B (Piyachaturawat et al., 2007).

7. Toxicity

Subchronic toxicity study

In 2003, Chivapat et al. investigated a subchronic toxicity of ethanolic extract of *C. comosa* rhizome in Wistar rats. Administration *C. comosa* ethanolic extract to rats at the doses of 100, 200, 400 and 800 mg/kg/day for 90 consecutive days. All doses of the extract did not affect body weight and food consumption of rats. At the dose of 800 mg/kg/day, *C. comosa* caused a significantly decrease of hematocrit, RBC and hemoglobin in male rats. Female rats receiving the extract at 200 mg/kg/day had a significant decrease of MCV and MCH but caused a significant increase of platelet number at 800 mg/kg/day of the extract. At this highest dose, the extract caused a significant increase of these following clinical blood chemistry parameters: ALP, ALT, total protein and albumin. Serum triglyceride was significantly decreased in male rats treated with 400 mg/kg/day and female rats treated with 200 and 400 mg/kg/day of the extracts. *C. comosa* extract at 200 mg/kg/day increased stomach

weight while other higher doses increased liver weight as well as hyperplasia and hyperkeratosis of gastric epithelium.

Biotransformation

Biotransformation or metabolism is one of the most important factors that can affect the overall therapeutic and toxic profile of a drug. It can lead to detoxification and excretion of the drug, but also to bioactivation resulting in toxicity. For this reason, drug biotransformation is a pivotal factor in the early developmental stage of new drugs.

Drug metabolism is normally divided into two phases: phase 1 (or functionalisation reaction) and phase 2 (or conjugation reaction). The main function of phase 1 metabolism is to prepare the compound for phase 2 metabolism. Phase 1 products are either less active or in many cases they are even more active than the parent compounds. Phase 2 is the true detoxification of drug which give products that are generally water soluble and easily excreted while in a lesser extent, phase 2 metabolism may yield a product of more active. Thus, drug metabolism can result in either a decreased or increased toxicity of the parent compound.

Phase I metabolism

Phase I metabolism includes oxidation, reduction, hydrolysis and hydration reactions, as well as other rarer miscellaneous reactions.

Oxidation involving cytochrome P450
(The microsomal mixed-function oxidase, MFO)

The mixed function oxidase system found in microsomes (endoplasmic reticulum) of many cells (notably those of liver, kidney, lung and intestine) performs many different functionalisation reactions. All of these reactions require the presence of molecular oxygen and NADPH as well as the complete mixed function oxidase system (cytochrome P450, NADPH-cytochrome P450 reductase and lipid). All reactions involve the initial insertion of a single oxygen atom into the drug molecule. A subsequent rearrangement and/or decomposition of this product may occur, leading to the final products seen. The MFO reaction is characterized by the following stoichiometry:

$$NADPH\ H^+ + O_2 + RH \longrightarrow NADP^+ + H_2O + ROH$$

Where RH represents an oxidisable drug substrate and ROH is the hydroxylated metabolite. The overall reaction is catalysed by the enzyme cytochrome P450. during the MFO reaction, reducing equivalents derived from NADPH H^+ are consumed and one atom of molecular oxygen is incorporated into the metabolite, whereas the other atom of oxygen is reduced to water.

Cytochrome P450

Cytochrome P450 (CYP) is a heam-containing enzyme (a haemoprotein), which exists as multiple forms of monomeric molecular weight of approximately 45000-55000 daltons. The heam of CYP is non-covalently bound to the apoprotein.

CYP proteins are categorized into families and subfamilies by their amino acid sequence similarities. Sequences that are greater than 40% identical at the amino acid level belong to the same family. Sequences that are greater than 55% identical are in the same subfamily. There are now more than 2500 cytochrome P450 sequences known. Table 2 gives an overview of the human CYP-families 1 to 4.

Table 2 The main human CYP isoforms (CYP1-4) involved in drug biotransformation and their occurrence. (Brandon et al., 2003)

Isoforms	Occurrence
CYP1A1	Mainly extrahepatic
CYP1A2	Liver
CYP2A6	Liver
CYP2B6	Liver
CYP2C8	Liver, intestine
CYP2C9	Liver, intestine
CYP2C18/2C19	Liver
CYP2D6	Liver, intestine
CYP2E1	Liver, intestine, leukocytes
CYP3A4	Liver, GI tract
CYP4A11	Liver, kidney

NADPH-cytochrome P450 reductase

NADPH-cytochrome P450 reductase, a flavin-containing enzyme, is a flavoprotein consisting of one mole of flavin mononucletide (FMN) and one mole of

flavin adenine nucleotide (FAD) per mole of protein. In addition to CYP, NADPH-cytochrome P450 reductase is an essential component of the MFO system responsible for drug oxidation in that the flavoprotein transfers reducing equivalents from NADPH H$^+$ to cytochrome P450 as

$$NADPH\ H^+ \longrightarrow FAD\ (NADPH\text{-}cytochrome\ P450\ reductase)FMN \longrightarrow CYP$$
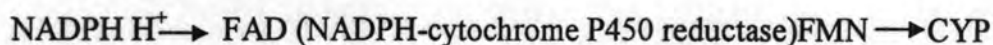
Lipid

Phosphatidylcholine is the lipid component, which is required for the MFO system. It has been suggested that lipid may either facilitate substrate binding, electron transfer or provide a template for the essential interaction of CYP and NADPH- cytochrome P450 reductase molecules.

Induction of drug metabolism

Role of CYP in the induction of drug metabolism

There are several drugs and chemicals capable of inducing hepatic metabolism. The induction of CYP plays a substantial role and has profound implications in clinical pharmacology and toxicology. These include drug tolerance, variability in drug response, drug-drug interaction, adverse drug reaction and drug toxicity.

The use of multiple drug therapy in the treatment of many diseases has led to problems with drug interactions. Many drug interactions are the result of interference of one drug with the metabolism of another and subsequently increase or decrease in the clearance of the latter drug.

Induction of drug metabolism may arise as a consequence of increase synthesis, decrease degradation, activation of pre-existing components or a combination of these three processes. However, it should be emphasized that the majority of CYP are induced at the level of transcriptional activation (Figure 2, Table 3).

synthesis
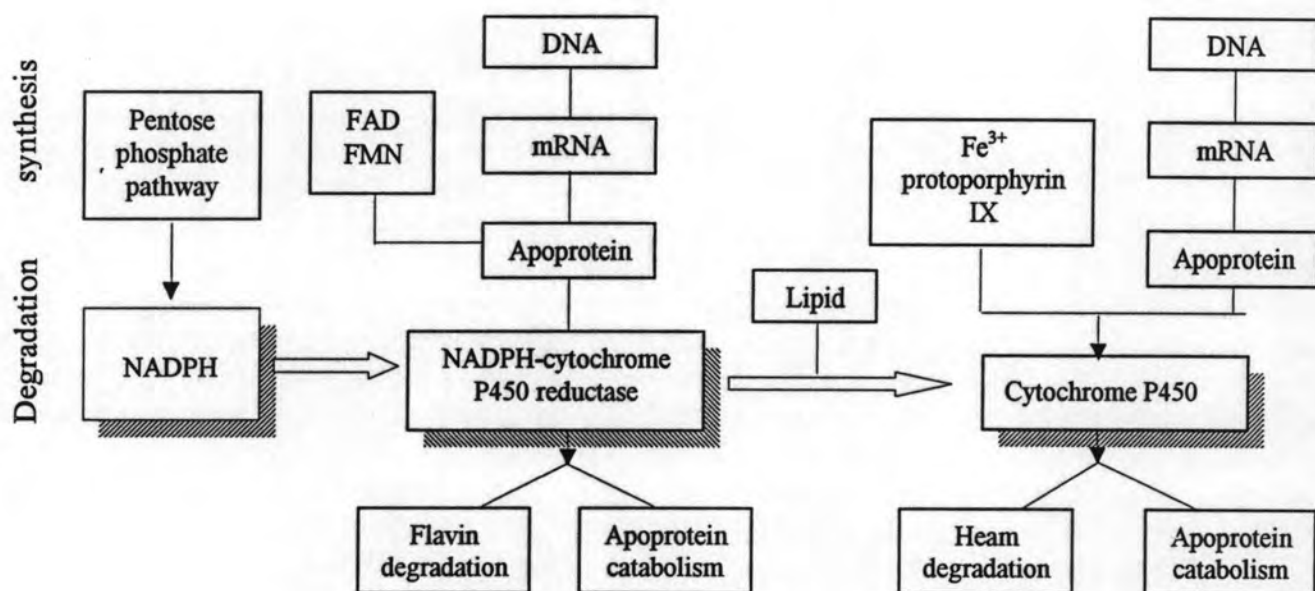
Degradation

Figure 2 Synthesis and degradation of functional components of the hepatic mixed-function oxidase system (Gibson and Skette, 2001)

Table 3 Differences in the induction mechanisms of CYP (Gibson and Skette, 2001)

CYP isoform	Representative inducer	Main induction mechanism
1A1	Dioxin	Transcription activation by ligand activated Ah receptor
1A2	3-Methylcholanthrene	mRNA stabilization
2B1/2B2	Phenobarbital	Transcriptional gene activation
CYP isoform	Representative inducer	Main induction mechanism
2E1	Ethanol, acetone, isoniazid	Protein stabilization (in part)
3A1	Dexamethasone	Transcriptional gene activation
4A6	Clofibrate	Transcriptional activation, mediated by peroxisome proliferators activated receptor

Molecular mechanisms of induction of CYP

CYP1A1

CYP1A1 binds and oxidizes planar aromatic, essentially flat molecules. These compounds are multiples of benzene, such as naphthalene and polycyclic aromatic hydrocarbons (PAHs). It is non-constitutive in the liver. This is probably because its natural function is not needed in the liver and an individual would not normally be exposed to large amount of planar aromatic hydrocarbons that might accumulate in the liver. However, the enzyme is found in other tissues, such as lung, where aromatics are more frequently encountered from traffic pollution and smoking.

CYP1A2

CYP1A2 originates from a gene on chromosome 15 in humans. It is linked with estrogen metabolism, as it is capable of oxidizing this series of hormones. Increased levels of this enzyme are also associated with colon cancer. CYP1A2 oxidizes planar aromatic molecules that contain aromatic amines, which its relative CYP1A1 does not. CYP1A2 orientates aromatic amines, some of which are quite large, in such a way as to promote the oxidation of amine group. Consequently, this enzyme is able to metabolize a variety of drugs that resemble aromatic amines. These include caffeine, β-naphthylamine (a known carcinogen) and theophylline. This enzyme is also capable of oxidizing estrogen. It tends to be inhibited by molecules that are planar, and possess a small volume to surface area ratio. It is blocked by the methylxanthine derivative furafylline.

The important ability of CYP1A1 is activate environmental compound such as the polycyclic aromatic hydrocarbons (PAHs) to biological reactive metabolites that interact with DNA, resulting in chemical carcinogenesis. CYP1A1 is induced by a large group of environmental chemicals as shown in the Table 4.

Table 4 Polycyclic aromatic hydrocarbon like inducers of CYP (Gibson and Skette, 2001)

Polycyclic aromatic hydrocarbons
3-methylcholantrene
Benzo[a]pyrene
Benzo[a]anthracene

Phenothiazines

β-naphthoflavone and other flavones

Plant indoles

  Indole-3-acetonitrite

  Indole-3-carbinol

Charcoal-broiled beef

Cigarette smoke

Crude petroleum

Polychlorinated biphenyls, polybrominated biphenyls

Halogenated dibenzo-p-dioxins

Halogenated dibenzofurans

Molecular mechanism of induction of CYP1A1/1A2

Polycyclic aromatic hydrocarbon inducers enter the cell and combine with the aryl hydrocarbon receptor or Ah receptor, a receptor complex in the cytoplasm which consists of a ligand-binding subunit and a heat-shock protein (HSP-90). The inducer-receptor complex translocates to the nucleus, leaving the heat-shock protein behind in the cytoplasm, and heterodimerises with the AhR nuclear translocator protein, termed Arnt. The inducer-AhR-Arnt complex binds to specific DNA sequences upstream of the CYP1A1 or 1A2 genes, which are termed xenobiotic responsive elements (XREs) or sometimes called drug responsive elements (DREs). An initiation complex thereby forms at the CYP1A1 promoter and increase the rate of CYP1A1 mRNA synthesis. Large amount of newly translated, specific CYP protein are then incorporated into the membrane of the hepatic endoplasmic reticulum (along with heam insertion), resulting in the observe induction of metabolism of certain drugs and xenobiotics. It should be noted that CYP1A1 is predominantly expressed in extra-hepatic tissue (particularly human lung) and is presented in low amounts in non-induced liver (approximately 2-5% of the total CYP content) but increases approximately 8-16 fold on induction, dependent on the species and tissue in question. CYP1A2 is constitutively expressed in human liver in low amounts.

CYP2B

CYP2B1 and CYP2B2 exhibit different rates of substrate biotransformation (CYP2B1 is usually more active than CYP2B2) and hence contribute to differential activation/deactivation of xenobiotic. In man CYP2B6 is the only member of the CYP2B family expressed in liver, whereas CYP2B7 is mainly expreesed in human lung. Typical inducers of these CYPs are phenobarbitone and related agents (Table 5).

Table 5 Drugs and chemicals that act as phenobarbitone-type inducers of CYP (Gibson and Skette, 2001)

| CYP2B inducers |
| --- |
| Phenobarbitone and several barbiturates |
| Phenyltoin |
| DDT |
| Pentamethylbenzene |
| Polychlorinated biphenyls (PCBs) with ortho chlorines |
| 2-Acetylaminofluorene |

Molecular mechanism of induction of CYP2B1/2B2

CYP 2Bs are controlled by a nuclear receptor called the constitutive androstane receptor (CAR), an unusual receptor in that it is transcriptionally active in the absence of bound ligand. Upon binding to its steroid ligands (aldrostanol [5α-androstan-3α-ol], androstenol [5α-androst-16-en-3α-ol]) is transcriptionally inactive. CAR is linked to a co-activator, SRC-1. CAR/SRC-1 complex will then bind to retinoic acid X-receptor (RXR) before it can bind DNA. The CAR/RXR complex binds to PBREM (phenobarbitone-responsive enhancer module) in the CYP2B gene. PBREM triggers induction by barbiturates (Sueyoshi et al., 1999). Although the precise molecular mechanisms whereby phenobarbitone interacts with this transcription factor complex still need clarifying, it would appear that the barbiturates (and other related inducers of the CYP2Bs) relieves the transcription repression of CAR produced by androstenol and androstranol. However, inducers probably cause CAR to bind SRC-1 even more tightly. Thus the expressions of these CYPs are closely controlled by a variety of different endogenous and exogenous agents.

CYP2E1

This CYP comprises around 7 percent of human liver CYP. It oxidizes small heterocyclic agents ranging pyridine through ethanol, acetone and other small ketones (methyl ethyl ketone). Ethanol and acetone are strong inducers of this isoform. Many of its substrates are water soluble and it is often implicated in toxicity, as the metabolites it forms can be highly reactive and toxic to tissues. It is responsible for oxidation of paracetamol. There is a polymorphism associated with this gene that is more common in Chinese people. The mutation correlates with a two-fold increase risk of nasopharyngeal cancer linked to smoking. Many sulphur-containing agents block this enzyme, such as carbon disulphide, diethyl dithiocarbamate and antabuse.

Molecular mechanism of induction of CYP2E1

Many of CYP2E1 substrates are bioactivated to reactive metabolites, including paracetamol and low molecular weight substrates such as nitrosamines, benzene, carbon tetrachloride and ethylene glycol, resulting in liver toxicity, kidney toxicity and carcinogen activation. Ethanol and other compounds such as imidazole, isoniazid, acetone and pyrazole are CYP2E1 inducers. It is not yet certain exactly how CYP2E1 is induced, but it appears that more than one mechanism is involved. When animals are exposed to CYP2E1 inducers, CYP2E1 protein levels are increased up to eightfold, although the CYP2E1 mRNA levels have not been seen to increase. This suggest that CYP2E1 is not induced like CYP1A1, rather that somehow the regulation is after transcription. Interestingly, the induction of CYP2E1 arises through multiple mechanisms depending on the induction stimulus and includes transcriptional, translational and post-translation mechanism. Whereas the predominant xenobiotic-dependent induction mechanism appears to be via stabilization. Presence of the substrate chemically stabilizes CYP2E protein and makes it functional, when it would normally be poorly or nonfunctional. The other mechanism is an inhibition of degradation of the CYP2E1 apoprotein (Figure 3).
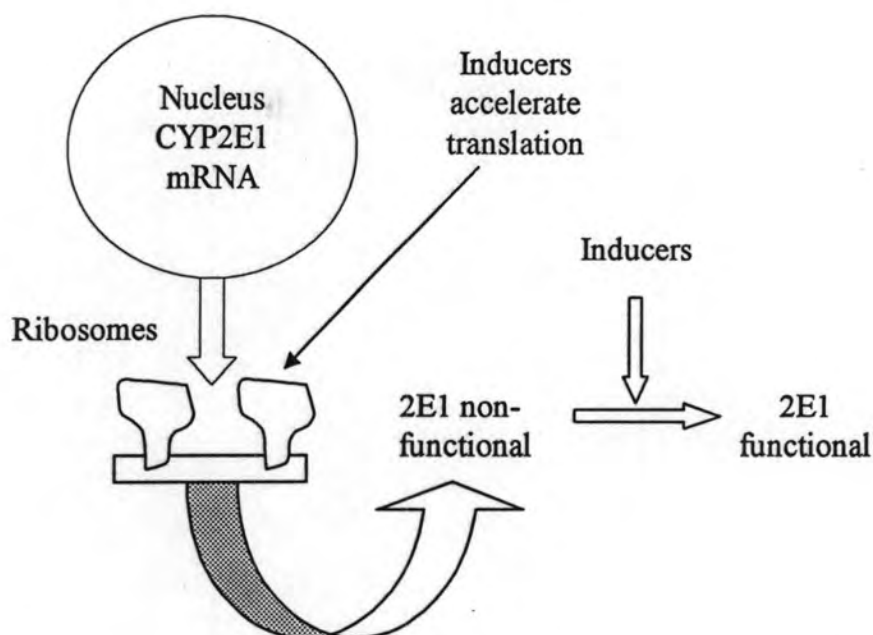
Figure 3 Proposed mechanism of CYP2E1 induction (Coleman, 2005)

CYP3A

The CYP3As subfamily are responsible for the metabolism of a wide range of drugs and other chemical in man and are highly inducible by drugs and both synthetic/natural steroids in liver (Table 10).

Currently, three human CYP3A enzymes have been identified, namely CYP3A4, CYP3A5, and CYP3A7. CYP3A4 is predominantly expressed in human liver and intestine. CYP3A4 plays a pivotal role in the metabolism of clinically used drugs and certain toxic environment chemical such as aflatoxin B_1.

CYP3A7 has only been detected in the liver and CYP3A5 appears to be constitutively express in the adult kidney.

Table 6 Substrates and inducers of human liver CYP3A4 (Gibson and Skette, 2001)

Substrates	Inducers
Aflatoxin B_1	Carbamazepine
Alfentanil	Dexamethasone
Codeine	Fexofenadine
Cyclophosphamide	Hypericum (St John's wort)
Cyclosporine	Lovastatin

Table 6 (con't) Substrates and inducers of human liver CYP3A4

(Gibson and Skette, 2001)

Substrates	Inducers
Dextrometrophan	Methylprednisolone
Diazepam	Metyrapone
Erythromycin	Phenobarbital
Lidocaine	Phenyltoin
Midazolam	Phenylbutazone
Nifedipine	Prednisolone
Omeprazole	Rifampicin
Ritonavir	Sulfinpyrazole
Tamoxifen	Spironolactone
Terfenadine	Troglitazone
Verapamil	Troleandomycin

Molecular mechanism of induction of CYP3A

Regulation of CYP3A gene is complex, mainly due to the plethora of regulation elements found in both the distal enhancer and proximal promoter (figure 3). The pregnane X receptor (PXR), plays an important role in CYP3A4 gene regulation for several xenobiotic and endogenous steroid inducers in several species include mouse, rat, rabbit and man. The PXR is a member of the nuclear receptor family, which found in the liver and intestine and may be part of an endocrine signaling pathway, the important structural features being a ligand (inducer)-binding domain and a DNA-binding domain. PXR is capable of binding and becoming activated by such a diverse group of inducers. The PXR/substrate complex binds to another nuclear receptor, the RXR (retinoic acid X receptor). The heterodimer binds to the response elements that are upstream of the human 3A4 gene, known as the ER6 elements. It has also been proposed that there is a second xenobiotic response element module (XREM) which is activated either as well as, or instead of, the ER6 elements. The expression of CYP3A4 is then upregurated according to the number of hits on the response element system. In addition, two further receptors mediate induction of the

CYP3As, the glucocorticoid receptor and the constitutive androsterone receptor (CAR).
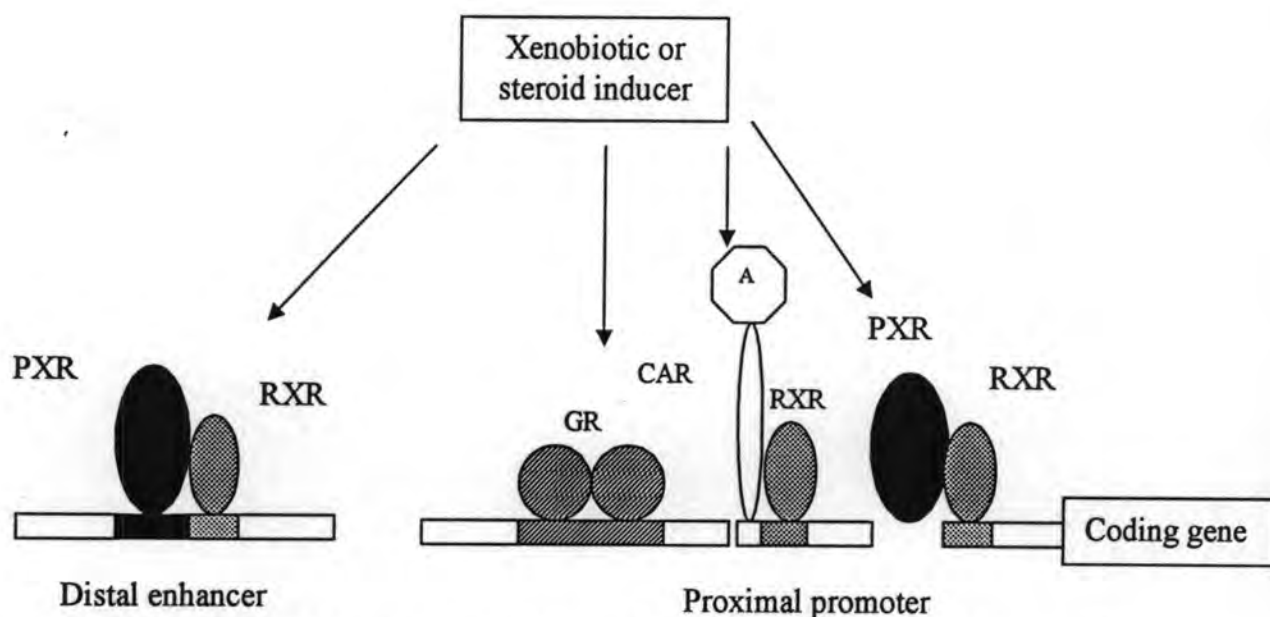
Figure 4 Receptor-dependent regulation of the CYP3A4 gene (Coleman, 2005) Abbreviations used: PXR, pregnane X receptor; RXR, retinoid X receptor; GR, glucocorticoid receptor; CAR, constitutive androstane receptor; A, constitutive CAR steroid ligands (androstanol and androstenol). Xenobiotic or steroid inducers can bind either comparative selectively to one of the receptor systems or promiscuously to more than one receptor with differing affinities. The receptor-inducer complex binds to specific regions in either the distal enhancer or proximal promoter modules, as dictated by the DNA-binding domain of the receptors.

Inhibition of drug metabolism

When two or more drugs are co-administered, a major concern is drug-drug interaction. Just as one drug can induce the metabolism of a second drug, the inhibition of drug metabolism by other drugs or xenobiotics is also a well recognized phenomenon. The inhibition of drug metabolism can take place in several ways, including the destruction of pre-existing enzymes, inhibition of enzyme synthesis or by complexing and thus inactivating the drug metabolizing enzymes. Commonly known selective inhibitors of the individual CYP are shown in Table 7.

Table 7 An overview of commonly known inhibitors of the CYP isozyme activity. (Brandon, 2003)

Isoforms	Inhibitors
CYP1A1	α-Naphtoflavone
CYP1A2	Furafylline
CYP2A6	Sulfaphenazole
CYP2B6	Sertraline
CYP2C8	Glitazones
CYP2C9	Sulfaphenazole
CYP2C18/2C19	Ticlopidine
	Ketoconazole
CYP2D6	Quinidine
	Haloperidol
CYP2E1	Diethyl-dithiocarbamate
CYP3A4	Ketoconazole
	Grapefruit juice
CYP4A11	17-Octadecynoic acid

Mechanism of CYP inhibition

Inhibition of CYP can be classified into 3 type as following: reversible inhibition, quasi-irriversible inhibition and irriversible inhibition (Lin and Lu., 1998).

1. **Reversible inhibition**

Reversible inhibition is the most common mechanism in drug-drug interaction. This inhibition occurs as a result of direct competition for the binding site on a CYP enzyme between a substrate and inhibitor (Yan and Coldwell, 2001). The competition can be either for the heme prosthetic group or for other region of the active site as lipophilic region of the protein. The potency of an inhibitor is determined both by its lipophilicity and by the strength of the bond between its nitrogen lone electron pair and the prosthetic heam iron. Many of potent reversible CYP inhibitors are nitrogen-containing drugs, including imidazoles, pyridines and quinolinesas. Examples of reversible inhibitors are shown in table 8. (Lin and Lu, 1998)

Table 8 Reversible CYP inhibitors (Lin and Lu, 1998)

CYP isoform	Drugs
CYP1A1/2	Ellipticine
CYP2C19	Omeprazole
CYP2D6	Quinidine
CYP3A4	Ketoconazole
	Cimetidine
	Indinavir

2. Quasi irreversible inhibition via metabolic intermediate complexation

This type of inhibition is caused by reactive metabolites generated from CYP-catalyzed reactions. These metabolites can form stable complexs with the prosthetic heam of CYP, called metabolic intermediate (MI) complex, so that the CYP is sequestered in a functionally inactive state. *In vitro* situation, MI complexation can be reserved, and the catalytic function of ferric CYP can be restored. However, *in vivo*, the MI complex is so stable that the CYP involved in the complex is unavailable for drug metabolism. Synthesis of new enzymes is the only way to restore activity (Lin and Lu, 1998). Inhibitors that act as quasi irreversible inhibition via metabolic intermediate complexation are shown (table 9).

Table 9 Drugs that act as quasi irreversible inhibition via metabolic intermediate complexation (Lin and Lu., 1998)

CYP isoforms	Drugs
CYP3A4	Erythromycin
CYP2B1	Orphenadrine
CYP2B1, CYP2C11, CYP3A1/2	Proadifen
CYP2C9	Isoniazid

3. Irreversible inactivation of CYP

Irreversible inactivation is caused by reactive intermediates. Drugs that can be oxidized by CYP to reactive intermediates, are classified as mechanism-based inactivators or suicide substrates. The inactivation of CYP may result from

irreversible alteration of heam or protein, or a combination of both. In general, modification of the haem group invariably inactivated the CYP, whereas protein alteration will result in loss of catalytic activity only if essential amino acids, which are vital for substrate binding, electron transfer and oxygen activation are modified (Lin and Lu, 1998).

Table 10 Drugs act as irreversible inhibitor (Lin and Lu, 1998)

CYP isoforms	Drugs
CYP3A4	Ethinylestradiol
CYP2B1,CYP2C6, CYP2C11	Chloramphenical
CYP2B1	2-ethynylnaphthalene

An impact of CYP induction and CYP inhibition of a xenobiotic on drug therapy, drug-drug interaction, adverse drug reaction and drug toxicity are mostly due to the modulation of CYP which is a particular enzyme involving in the metabolism of the drug substrate. Currently used medicines which are substrates of individual isoform of CYP are shown in Table 11.

Table 11 Drugs which are substrates of the individual CYP (Cupp and Tracy, 1998)

Isoforms	Substrate
CYP1A2	Amitriptyline
	Clomipramin
	Clozapine .
	Imipramine
	Propranolol
	R-warfarin
	Theophyiline
	Tacrine
CYP2B6	Bupropion
	Cyclophosphamide
	Efavirenz
	Ifosfamide

Table 11 (con't) Drugs which are substrates of the individual CYP (Cupp and Tracy, 1998)

Isoforms	Substrate
CYP2E1	Acetaminophen
	Ethanol
CYP3A	Amitriptyline
	Benzodiazepines
	Alprazolam
	Triazolam
	Midazolam
	Calcium blockers
	Carbamazepine
	Cisapride Dexamethasone
	Erythromycin
	Ethinyl estradiol
	Glyburide
	Imipramine
	Ketoconazole
	Lovastatin
	Nefazodone

Modulation of CYP is also contributed to an increase or decrease human risks to environmental chemical toxicities. Examples of environmental chemicals that are metabolic activated by each individual CYP are shown in Table 12.

Table 12 Protoxicants and procarcinogens activated by human P450s
(Guengerich et al., 1992)

Isoforms	Protoxicants and procarcinogens
CYP1Al	Benzo[a]pyrene and other polycyclic hydrocarbons 2-Amino-l-methyl-6-phenylimidazo[4,5-b] pyridine
CYP1A2	2-Acetylaminofluorene 2-Aminofluorene 2-Aminoanthracene 2-Amino-3-methylimidazo[4,5-pquinoline (IQ) 2-Amino-3,8-dimethylimidazo[4,5-fquinoline (MeIQ) 2-Amino-3,8-dimethylimidazo[4,5-flquinoxaline (MeIQx) 2-Amino-3,4,8-trimethylimidazo[4,5-]quinoxaline (DiMeIQx) 2-Amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu P-1) 3-Amino-1-methyl-5H-pyrido[4,3-b]indole (Trp P-2) 2-Aminodipyrido[1,2-a:3',2'-d]imidazole (Glu P-2) 2-Naphthylamine 4-Aminobiphenyl 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) 4-N-Nitroso(methylamino)-1-(3-pyridyl)-

Table 12 (con't) Protoxicants and procarcinogens activated by human P450s
(Guengerich et al., 1992)

Isoforms	Protoxicants and procarcinogens
CYP2E1	N-Nitrosodimethylamine
	N-Nitrosodiethylamine
	N-Nitroso-N-methylbenzylamine
	N-Nitrosomethylbutylamine
	Benzene
	Carbon tetrachloride
	Chloroform
	Methylene chloride
	Trichloroethylene
	Ethylene dichloride
	Styrene
	Vinyl chloride
	Vinyl bromide
	Acrylonitrile
	Ethyl carbamate
	Vinyl carbamate
CYP3A4	Aflatoxin B1
	Aflatoxin G1
	Sterigmatocystin
	Senecionine
	9,10-Dihydroxy-9,10-dihydrobenzo[b]fluoranthene
	3,4-Dihydroxy-3,4-dihydro-7,12-dimethylbenz[a]anthracene
	6-Aminochrysene