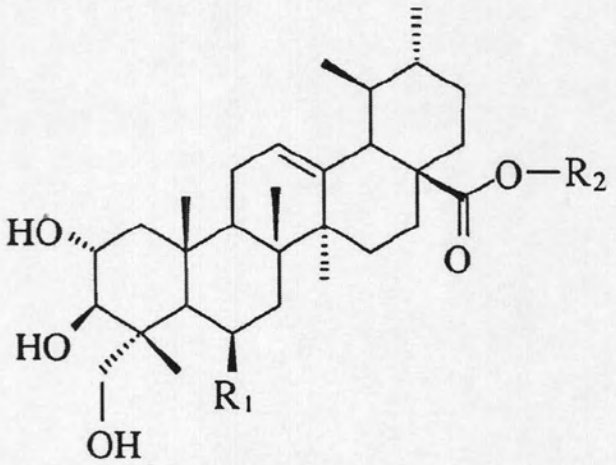
CHAPTER II

LITERATURE REVIEWS

Centella asiatica (Linn.) Urban is a member of the Umbelliferae family. It is a perennial herb, herbaceous creeper with fan shaped leaves (Figure 1). This plant is found throughout in moist places up to altitude of 1,800 m. It processes long, brownish, string shaped stolons that are characterized by long internodes with roots at each node. It reproduces both through vegetative and sexual means, The seed are very small in size (Singh and Singh, 2002). It is known as Mandukparni, Indian pennywort, Asiatic pennywort, Gotu kola in English name and a common name in Thai as Bua bok. This plant is found almost all over the world including in China, India, Southeast Asia, Sri Lanka, Africa and Oceanic countries. The whole plants of *C. asiatica* has been used for centuries in traditional medicine for the treatment of skin diseases, wounds, cardiotonic, diruretic etc.

C. asiatica contains various constituents. Main active compounds of this plant are triterpenoid compounds, principally asiatic acid, madecassic acid, asiaticoside and madecassoside (Inamdar et al., 1996; Randriamampionona et al., 2007). The structure of these constituents was shown in Figure 2.

Figure 1 *Centella asiatica* (Linn.) Urban



Asiatic acid	$R_1 = H$	$R_2 = H$
Madecassic acid	$R_1 = OH$	$R_2 = H$
Asiaticoside	$R_1 = H$	R_2 = Glu-Glu-Rha
Madecassoside	$R_1 = OH$	R_2 = Glu-Glu-Rha

Figure 2 Structure of the *C. asiatica* compounds (Randriamampionona, et al., 2007)

Pharmacological effects

1. Wound healing effect

Many studies have revealed that the extract of *C. asiatica* have significant wound healing activity (Shetty et al., 2006; Shukla et al., 1999).

Shetty et al. (2006) studied effect of ethanolic extract of *C. asiatica* in both normal and dexamethasone suppressed wound healing, using incision, excision, and dead space wounds models in Wistar albino rats. The results indicated that the extract promotes wound healing significantly and is able to overcome the wound-healing suppressing action of dexamethasone in a rat model.

Activity of asiaticoside, isolated from *C. asiatica*, had been studied in normal as well as delayed-type wound healing. It was shown to produce 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and better epithelisation in guinea pig punch wounds that topical applied of 0.2% solution of asiaticoside. In streptozotocin diabetic rats, where healing is delayed, topical application of 0.4% solution of asiaticoside over punch wounds found an increases in hydroxyproline content, tensile strength, collagen content and epithelisation there by facilitating the healing. Asiaticoside was also active by the oral route at 1 mg/kg dose in the guinea pig punch wound model. It also promoted angiogenesis in the chick chorioallantoic membrane model at 40 mg/disk concentration (Shukla et al., 1999).

2. Protective effect on gastric ulcer formation

Effect of *C. asiatica* extract on the prevention of ethanol induced gastric lesions was investigated in rats. It was shown that gastric transmucosal potential difference reduced by the application of 50% ethanol in the gastric ex-vivo chamber model. Orally administration of *C. asiatica* (0.05 g/kg, 0.25 g/kg and 0.50 g/kg) before ethanol administration significantly inhibited gastric lesions formation (58% to 82% reduction) and decreased mucosal myeloperoxidase activity in a dose dependent manner (Cheng and Koo, 2000).

Cheng et al. (2004) have reported that orally administration of *C. asiatica* extract and asiaticoside, an active constituent of the extract in rats reduces the size of the ulcers at day 3 and 7 in a dose-dependent manner, with a concomitant attenuation of myeloperoxidase activity at the ulcer tissues. Epithelial cell proliferation and angiogenesis were on the other hand promoted. The expression of basic fibroblast

growth factor, an important angiogenic factor, was also upregulated in the ulcer tissues in rats treated with the extract or asiaticoside.

3. Learning and memory enhancing effect

Aqueous extract of *C. asiatica* (200 mg/kg for 14 days) showed an improvement in learning and memory in both shuttle box and step through paradigms in male Wistar rats (Kumar and Gupta, 2002).

Gupta et al. (2003) suggested that administration of *C. asiatica* (300 mg/kg orally) decrease the Pentylenetetrazole (PTZ)-kindled seizures. An improvement in the learning deficit induced by PTZ kindling was shown as evidenced by decreased seizure score and increased latencies in passive avoidance behavior in male Wistar rats that were injected PTZ (30 mg/kg, i.p.) once every alternate day (48 $\pm$ 2 h) until the development of the kindling. However, low dose of the *C. asiatica* (100 mg/kg) was shown to improve only in the learning deficit due to the kindling and failed to improve the seizure score.

Orally administration of *C. asiatica* (200 mg/kg) for 15 days from day 15 to day 30 post partum (p.p.) was shown to pass the radial arm maze to assess the learning and memory performance. This dose was selected for nootropic studies on the 31st day and 6 months p.p. It was found that performance of juvenile and young adult mice was significantly improved in radial arm maze and hole board tests. Treatment resulted in increased acetylcholine esterase activity in the hippocampus. Dendritic arborization of hippocampal CA3 neurons was also increased in terms of intersections and branching points, both at one month and 6 months (Rao et al., 2005).

The neonatal rat pups (7 days old) treated with 4 and 6 ml/kg body weight per day of fresh leaf extract of *C. asiatica* for 4 and 6 weeks were shown to significantly increase in the dendritic length (intersections) and dendritic branching points along the length of both apical and basal dendrites. This study concluded that the constituents/active principles present in *C. asiatica* fresh leaf extract had a neuronal dendritic growth stimulating property; hence, the extract can be used for enhancing neuronal dendrites in stress and neurodegenerative and memory disorders (Rao et al., 2006).

4. Antioxidant effect

Hamid et al. (2002) found that the ethanolic extract of all parts of *C. asiatica* exhibited significantly higher antioxidative activity than the water extract.

Treatment of the aqueous extract of whole plant of *C. asiatica* (200 and 300 mg/kg) in male Wistar rats showed a significant decrease in the brain levels of malondialdehyde with simultaneously significant increase in the level of glutathione. There was a significant increase in the levels of catalase at the 300 mg/kg of the aqueous extract of *C. asiatica* (Kumar and Gupta, 2002).

Zainol et al. (2003) reported that both leaf and root of *C. asiatica* prossessed antioxidant activities. Oral treatment with 50 mg/kg/day of crude methanolic extract of *C. asiatica* for 14 days significantly increased the anti-oxidant enzymes, like superoxide dismutase, catalase and glutathione peroxidase, and anti-oxidants like glutathione and ascorbic acid decreased in lymphoma-bearing mice (Jayashree et al., 2003).

Rats administered with adriamycin (2.5 mg/kg body wieght, i.p.) caused myocardial damage that was manifested by the elevation of serum marker enzymes such as lactate dehydrogenase, creatine phosphokinase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, and showed significant changes in the antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase, glutathione *S*-transferase. Pre- and/or co-treatment with *C. asiatica* extract (200 mg/kg of body weight, orally) extract significantly prevented these alterations and restored the enzyme activities to near normal levels (Gnanapragasam et al., 2004).

Hussin et al. (2007) concluded that *C. asiatica* extract may ameliorate H_2O_2-induced oxidative stress by decreasing lipid peroxidation via alteration of the antioxidant defence system of the rats that treated with H_2O_2 (0.1%) for 25 week.

Aqueous extract of *C. asiatica* had the propensity to modulate both endogenous and neurotoxicant induced oxidative impairments in the brain in male mice given *C. asiatica*-incorporated diet (0.5% and 1.0%) for 4 weeks. There was significant diminution in the levels of malondialdehyde (30-50%), reactive oxygen species (32-42%) and hydroperoxide levels (30-35%), which was accompanied by enhanced activities of antioxidant enzymes in all brain regions. While the levels of reduced glutathione and total thiols were elevated, the protein carbonyl content was decreased in brain among *C. asiatica*-fed mice. Further, the aqueous extract of

C. asiatica showed significant free radical scavenging activity determined in established chemical test systems (viz., 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide and hydroxyl radical scavenging activity) (Shinomol and Muralidhara, 2008).

5. Antitumor activity

Anti-tumour effect of the crude extract of *C. asiatica* showed 100% cytotoxicity at the concentration of 100 µg/ml and the concentration required to produce 50% cell death was 62 µg/ml of concentration for Ehrlich ascites tumour cells and 75 µg/ml of concentration for Dalton's lymphoma ascites tumour cells. Oral administration of crude extract of *C. asiatica* significantly reduced the development of murine solid tumour and increased the life span of tumour bearing mice (Babu et al., 1995).

Effects of the water extract of *C. asiatica* on formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and intestinal tumorigenesis in male F344 rats have been investigated. It was shown that treatment with the extract significantly decreased the number of larger ACF (with four or more crypts per focus) in the large intestine in the early stage, while the number of methylated DNA adducts was not decreased compared to that in the AOM-treated group. In the post-initiation stage, the extract significantly decreased the total number of ACF and the number of larger ACF, accompanied by a decrease in the 5-bromo-20-deoxyuridinelabeling index and an increase in the induction of apoptotic cells in the colonic mucosa. The incidences of neoplasms, the numbers of adenocarcinomas in the small intestines and entire intestines, and sizes of neoplasms in the entire intestines in rats fed *C. asiatica* extract at a dose of 10 mg/kg were smaller than those in rats given AOM alone. The extract at a dose of 100 mg/kg significantly reduced the multiplicity of neoplasms in the small intestine (Bunpo et al., 2004).

Antigenotoxic effect of *C. asiatica* extract was studied against the genotoxic effect induced by cyproterone acetate (CPA) on human lymphocytes using chromosomal aberrations and sister chromatid exchanges as parameters. It was shown that treatment of the two doses of CPA, i.e. 20 and 30 µM along with *C. asiatica* extract at the concentrations of 1.075×10^{-4}, 2.125×10^{-4}, 3.15×10^{-4} and 4.17×10^{-4} g/ml of the culture medium decreased in the genotoxic damage of CPA in

a concentration dependent manner. This suggested a protective role of *C. asiatica* extract again CPA-induced genotoxic effect (Siddique et al., 2008).

6. Immunological activity

S3A was a RG-I pectin isolated from *C. asiatica* that contained Rha, Ara, Gal, Glc and GalA in a molar ratio of 1.0: 0.6: 1.5: 0.2: 1.1 and had been found to have a backbone composed mainly of the disaccharide repeat unit. The pharmacological of S3A derivatives showed remarkable immuno-enhancing activity. Among the samples, S3A-N enhanced the proliferation of B lymphocytes much more remarkably than that shown by T-lymphocytes, and S3A-L, S3A-I, S3A-S and S3AP3 had similar proliferative effects on both T- and B-lymphocytes (Wang et al., 2003).

Jayathirtha and Mishra (2004) reported that methanolic extract of *C. asiatica* (contained 0.18% asiaticoside) significantly increased the phagocytic index at the doses of 100 to 500 mg/kg/body weight. Total white blood cell count was also significantly increased at the dose of 500 mg/kg/body weight.

Pectin was extracted from *C. asiatica*, and its degraded product showed immuno-stimulating activity in a different extent *in vitro*. It was shown that 1BIIP had some proliferation enhancement in the concentration of 100 mg/ml on T and B lymphocytes. These results suggested that the galactosyl or arabinosyl side chains of pectin had effect on the expression of immunological activity (Wang et al., 2005).

Punturee et al. (2004) suggested that water extract of *C. asiatica* could either increase or decrease nitric oxide (NO) production by J744.2 mouse macrophages and these effects were predominantly mediated through an effect on tumour necrosis factor-α (TNF-α) expression.

Clinical studies

Randomized, double-blind, placebo-controlled study of the effects of *C. asiatica* (750 mg once daily for 2 months) on cognitive function and mood disorder of twenty-eight healthy elderly volunteers were investigated. Cognitive performance was assessed using the computerized test battery and event-related potential whereas mood was assessed using Bond-Lader visual analogue scales prior to the trial and after single, 1 and 2 months after treatment. The results showed that the plant extract enhanced working memory and increased N100 component amplitude of event-

related potential. Improvements of self-rated mood were also found following the *C. asiatica* treatment (Wattanathorn et al., 2008).

Case report showed that three women (61, 52 and 49 years old) developed jaundice after taking *C. asiatica* (for 30, 20 and 60 days, respectively. The laboratory tests showed as following: alkaline phosphates: 1193, 1694 and 324 U/L; alanine aminotransferase: 503, 472 and 484 U/L; bilirubin: 4.23, 19.89 and 3.9 mg/dl. The first patient also had anti-smooth muscle antibodies 1/160 and anti-mitochondrial antibodies 1/320. Also, the pathological diagnoses showed as following: granulomatous hepatitis with marked necrosis and apoptosis; chronic hepatitis with cirrhotic transformation and intense necroinflammatory activity; and granulomatous hepatitis, respectively. Thus, *C. asiatica* should be taken into account as a potential etiology of hepatic injury associated with apoptosis and necrosis (Jorge and Jorge, 2005).

Xenobiotic biotransformation

Xenobiotic biotransformation or metabolism is a metabolic process that alters the chemical structure of xenobiotics, which are foreign compounds lead to termination or alteration of biological activity. In general, enzymatic metabolism transforms lipophilic parent compound to more hydrophilic metabolites, which can be readily excreated into bile or urine, but also to bioactivation resulting in toxicity. For this reason, drug biotransformation is a hinge factor in the early developmental stage of new drugs.

Drug metabolism is generally classified into two groups, called phase I (or functionalisation reactions) and phase II (or conjugation reactions), as shown in Table 1. The major function of phase I metabolism is to prepare the compound for phase II metabolism. Phase I metabolism generally results in the introduction of functional group into molecules or exposure of new functional groups of molecules, whereas phase II metabolism involves in the detoxification of drug which give products that are generally water soluble and easily excreted. Phase II metabolism may yield a product of more active. Thus, drug metabolism can result in either a decreased or increased toxicity of the parent compound.

Table 1 General pathways of xenobiotic biontransformation and their major subcellular location (Gibson and Skett, 2001; Ioannides, 1996; Parkinson, 2001)

Reaction	Enzyme	Localization
	Phase I	
Oxidation	Mixed-function oxidases	Microsomes
	Monoamine oxidases	Mitochondria
	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Mitochondria,Cytosol
	Aldehyde oxidases	Cytosol
	Xanthine oxidase	Cytosol
	Diamine oxidase	Cytosol
	Prostaglandin H synthase	Microsomes
	Flavin-monooxygenases	Microsomes

Table 1 (con't) General pathways of xenobiotic biontransformation and their major subcellular location (Gibson and Skett, 2001; Ioannides, 1996; Parkinson, 2001)

Reaction	Enzyme	Localization
	Phase I (con't)	
Reduction	Azo- and nito-reductase	Microflora, microsomes, cytosol
	Carbonyl reductase	Cytosol, blood, microsomes
	Disulfide reductase	Cytosol
	Sulfoxide reductase	Cytosol
	NAD(P)H -quinone oxidoreductase	Cytosol, microsomes
	Reductive dehalogenatase	Microsomes
Hydrolysis	Esterases	Microsomes, cytosol, lysosomes, blood
	Pepitdase	Blood, lysomes
	Epoxide hydrolase	Microsomes, cytosol
	Phase II	
Glucuronide conjugation	UDP-glucuronosyltransferase	Microsomes
Sulfate conjugation	Sulfotransferase	Cytosol
Glutathione conjugation	Glutathione *S*-transferase	Cytosol, microsomes
Methylation	*N*- and *O*-methyl transferase	Cytosol, microsomes, blood
Acylation	*N*-acetyl transferase	Mitocondria, cytosol
Amino acid conjugation	Acyl-CoA:amino acid *N*-acyltransferse	Mitocondria, microsomes

Phase I metabolism

Phase I metabolisms involve oxidation, reduction, hydrolysis and hydration reactions of drugs or xenobiotics, as well as other rarer miscellaneous reactions. This

reactions expose or introduce a hydrophilic functional groups (-COOH, -NH_2, -SH, or -OH).

The microsomal mixed-function oxidase (MFO)

The mixed function oxidase system found in endoplasmic reticulum (microsome) 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 or xenobiotic molecule. A subsequent rearrangement and/or decomposition of this product may occur, leading to the final products seen. The general stoichiometry of MFO reaction is characterized as following:

$$NADPH\ H^+ + O_2 + RH \xrightarrow{\text{Cytochrome P450}} NADP^+ + H_2O + ROH$$

where RH represents an oxidisable drug substrate and ROH is the hydroxylated metabolite, the overall reaction being catalysed by the cytochrome P450 enzymes. During the 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

All cytochrome P450 (CYP) enzymes are haem-containing proteins, which exists as multiple forms of monomeric molecular weight of approximately 45000-55000 daltons. The haem of CYP is non-covalently bound to the apoprotein and the name 'cytochrome P450' is derived from the fact that cytochrome exhibits a spectral absorbance at 450 nm when reduced (Fe^{2+}-haem) and complexed with carbon monoxide.

CYPs are classified 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 and the same subfamily shares at 55% amino acid sequence. Over 57 human cytochrome P450 have now been isolated, unequivocally identified and characterized to date, including the major human liver drug-metabolizing cytochrome P450 (CYP1A2, CYP2E1, CYP2C9, CYP2C19, CYP2D6

and CYP3A4) and the steroid hydroxylase including CYP1A2 and CYP21A2. The main human CYP isoforms involved in drug biotransformation and their occurrence are shown in Table 2.

Table 2 The main human CYP isoforms involved in drug biotransformation and their occurrence (Gibson and Skett, 2001; Guengerich, 2005)

Isoforms	Tissue sites	Subcellular localization
CYP1A1	Liver, peripheral blood cell	Microsomes
CYP1A2	Liver	Microsomes
CYP2A6	Liver	Microsomes
CYP2B6	Liver, lung	Microsomes
CYP2C8	Liver	Microsomes
CYP2C9	Liver	Microsomes
CYP2C18/2C19	Liver	Microsomes
CYP2D6	Liver	Microsomes
CYP2E1	Liver, lung, other tissues	Microsomes
CYP3A4	Liver, small intestine	Microsomes
CYP3A5	Liver, lung	Microsomes
CYP4A11	Liver	Microsomes

The main human CYP isoforms

CYP1A2 originates from a gene on chromosome 15 in humans. It is linked with oestrogen metabolism, as it is capable of oxidizing this series of hormones. This enzyme is able to metabolize a variety of drug that resemble aromatic amines: these include caffeine, β-naphthylamine (a known carcinogen), theophylline etc. It is also capable of oxidizing oestrogens and it is blocked by methylxanthine, α-naphthoflavone etc.

CYP2B6 originates from a gene found on chromosome 19, is the only CYP2B form found in man. This isoform oxidize amfebutamone (bupropion), mephenytoin, some coumarins, cyclophosphamide and its derivatives, as well as methadone. Orphenadine and miconazole are inhibitors of this CYP (Coleman, 2005).

CYP2C9 is absent in fetal liver and is also expressed in the small intestine (Guengerich, 2005). The recent crystallization of CYP2C9 has informed a great deal

about its structure as related to function. It has evolved to process relatively small, acidic and lipophilic molecules. There are large numbers of substrates for this CYP, which include tolbutamide, dapsone, warfarin etc. The active site is large and it appears that there is more than one place where drugs can bind. Sulfaphenazole is a highly selective inhibitor of this enzyme.

CYP2C19 is also inducible and differs by only around 10 percent of its amino acids from 2C9. But it does not oxidize acidic molecules, indicating that the active sites and access channels are subtly different. This CYP metabolizes omeprazole, a common ulcer drugs. Tranylcypromine and miconazole acts as a potent inhibitor of CYP2C19.

CYP2D6 is responsible for more than 70 different drug oxidations. It is found a little in the gut, and comprises about 2-4 percent of the CYPs in human liver. Several groups of drugs are metabolized by this enzyme, including antiarrhythmics (flecainide, mexiletine), tricyclic antidepressant (amitriptyline), selective serotonin reuptake inhibitor (SSRIs) and related antidepressants (paroxetine, fluoxetine), antipsychotics (chlorpromazine, haloperidol), beta-blockers (propanolol, labetalol, timolol) and analgesics (codeine, fentanyl). Quinidine inhibits this enzyme.

CYP2E1 comprises around 7 percent of human liver P450. It is unusual as a mammalian CYP in that it oxidizes small heterocyclic agents, ranging from pyridine through ethanol, acetone and other small ketones (methyl ethyl ketone). Many 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 such as paracetamol. Sulphur-containing agents block this enzyme, such as diethyl dithio carbamate. Imipramine also inhibits this CYP.

CYP3A4 is responsible for the metabolism of more than 120 drugs and comprises around 28 percent of total CYP. It is found in liver and intestinal walls. The major function is to metabolize steroids. It active site is so large and flexible, which its evolution to oxidize such a wide variety of substrates such as cyclosporine A (bulky molecules), paracetamol (small molecules). Azole antifungal agents such as ketoconazole and fluconazole, as well as anti-HIV agents such as ritronavir, inhibit CYP3A4 (Coleman, 2005).

Inhibition of drug metabolism

The drug-drug interactions are a great concerned, when two or more drugs are co-administered. 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 by drug or xenobiotics 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. Thus, the inhibition of CYP is of clinical importance for both therapeutic and toxicological reasons.

Mechanism of CYP inhibition

Mechanism of CYP inhibition can be divided into three types (Lin and Lu., 1998).

1. **Reversible inhibition**

Reversible inhibition is probably the most common mechanism responsible for the documented drug interactions. This inhibition occurs as a result of direct competition for the binding site on a CYP enzyme between a substrate and inhibitor. The effect of this inhibition will be dissipated after discontinuing the inhibitor.

2. **Quasi irreversible inhibition via metabolic intermediate complexation**

Quasi irreversible inhibition occurs when a reactive metabolite form a stable complex with prothetic heme of CYP. The stable complex is called metabolic intermediate (MI) complex. As a result, CYP is sequestered in a functionally inactive state. MI complexation can be reserved, and the catalytic function of ferric CYP can be restored by an *in vitro* situation. However, the MI complex is so stable that the CYP involved in the complex is unavailable for drug metabolism by an *in vivo* situation. Synthesis of new enzymes is the only way to restore activity (Lin and Lu, 1998).

3. **Irreversible inactivation of CYP**

Irreversible inactivation is caused by reactive intermediates. Drugs or xenobiotics that can be oxidized by CYP to reactive intermediates, are classified as mechanism-based inactivators or suicide substrates. The intermediates are able to be bind with the components of CYP included a pyrrole nitrogen in prosthetic heme group, heme moiety and apoprotein. The result is inhibiting the binding of substrate to enzyme and the inhibition is usually irreversible (Lin and Lu, 1998).

The impact of CYP inhibition on drug therapy, drug-drug interaction, adverse drug reaction and drug toxicity are due to the modulation of CYP which is a particular enzyme involving in the metabolism of the drug substrate. Example of substrate, inhibitor and inducer of the human CYP isoform involved in xenobiotic biotransformation are shown in Table 3.

Table 3 Examples of substrates, inhibitors and inducers of the human CYP isoforms involved in xenobiotic biotransformation (Coleman, 205; Dangles and Dufour, 2006; Parkinson, 2001)

Isoforms	Substrates	Inhibitors	Inducers
CYP1A2	Amitriptyline Clomipramin Clozapine Imipramine Propranolol Theophylline Galangin	Ciprofloxacin Fluovoxamine Furafylline Mehylxanthine α-Naphthoflavone	Charcoal- broiled beef Cigarette smoke Cruciferous vegetables Omeprazole
CYP2B6	Bupropion Cyclophosphamide Efavirenz Ifosfamide *S*-Mephenyltoin	Methoxychlor Orphenadrine Miconazole	Phenobarbital Phenyltoin Rifampicin Troglitazone
CYP2C9	Celecoxib Diclofenac Phenobarbital Phenyltoin Tolbutamide	Sulfaphenazole Sulfinpyrazone	Rifampicin
CYP2C19	Diazepam Lansoprazole Omeprazole Pentamidine Phenobarbital Flavonoids	Fluconazole Miconazole Teniposide Tranylcypromine	Rifampicin

Table 3 (con't) Examples of substrates, inhibitors and inducers of the human CYP isoforms involved in xenobiotic biotransformation (Coleman, 2005; Dangles and Dufour, 2006; Parkinson, 2001)

Isoforms	Substrates	Inhibitors	Inducers
CYP2D6	Amitriptyline Captopril Chlorpramazine Dextromethorphan Flunarizine Fluphenazine Propanolol Tamoxifen	Cerecoxib Chinidin Fluxetine Lobelin Propidin Quinidine Trifluperidol Miconazole	Piperidine Carbamazepine Dexamethasone Rifampicin
CYP2E1	Acetaminophen Alcohol Caffeine Dapsone Theophylline	Diethyldithiocarbamate Dimethyl sulfoxide Disulfiram Imipramine 4-Methylpyrazole	Ethanol Isoniazid
CYP3A	Amitriptyline Benzodiazepines Alprazolam Triazolam Midazolam Calcium blockers Carbamazepine Cisapride Erythromycin Ethinyl estradiol Glyburide Imipramine Ketoconazole Lovastatin Nefazodone	Amprenavir Clotrimazole Fluoxetine Indinavir Itraconazole Ketoconazole Miconazole Nicardipine Saquinavir Verapamil α-Naphthoflavone	Carbamazepine Dexmethasone Glutethimide Nevirapine Phenobarbital Phenyltoin Rifambutin Rifampicin Sulfadimidine St. John's Wort Sulfadimidine Troglitazone Troleandomycin

Biotransformation does not always lead to detoxification. It is also contributed to an increase or decrease human risks to environmental chemical toxicities or tumorigenicity. Examples of environmental chemicals that are metabolic activated by each individual human CYP is summarized in Table 4.

Table 4 Procarcinogens and protoxicants activated by human CYPs (Guengerich, 2005; Parkinson, 2001)

Isoforms	Procarcinogens and protoxicants
CYP1A2	Acetaminophen 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-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) Tacrine
CYP2B6	6-Aminochrysene Cyclophosphamide Ifosphamide

Table 4 (con't) Procarcinogens and protoxicants activated by human CYPs (Guengerich, 2005; Parkinson, 2001)

Isoforms	Procarcinogens and protoxicants
CYP2C9, CYP2C19	Tienilic acid Valproic acid
CYP2D6	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
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

Phase II metabolism

The major phase II reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione and conjugation with amino acid.

Glucuronidation

Glucuronidation is the addition (conjugation) of glucuronic acid in an activated form as UDP-glucuronic acid (UDPGA) to functional groups such as hydroxyl, amine, sulfhydryl and carboxylic acid. This reaction catalyze by UDP-glucuronosyltransferase enzymes (UDPGT) (Zhang et al., 2007). UDPGT can directly conjugate drugs without the prerequisite of phase I metabolism. The reaction of glucuronide conjugation as following:

$$\text{R-OH} + \text{UDPGA} \longrightarrow \text{R-OGA} + \text{UDP}$$

$$\text{R-COOH} + \text{UDPGA} \longrightarrow \text{R-COOGA} + \text{UDP}$$

$$\text{R-NH}_2 \text{ (or SH)} + \text{UDPGA} \longrightarrow \text{R-NH (or S) GA} + \text{UDP}$$

where R-OH, R-COOH and R-NH_2 (or SH) are xenobiotic or endobiotic and UDPGA is UDP-glucuronic acid. This reaction is catalysed by a family of enzymes, UDPGTs. The additions of glucuronic acid results in conjugates that are more polar, ionized at physiologic pH and have an increase molecular weight. These features facilitate excretion of glucuronides via the kidney either by glomerular filtration or by active secretion or both. In addition, glucuronides are commonly excreted by the liver via the bile into the small intestine and they are too polar to diffuse through cell membrane (Zhang et al., 2007).

UDPGT is present in many tissues, mostly in liver, but also in the kidney, small intestine, lung, skin, adrenals and spleen. The active site of the UDPGT faces the lumen of the endoplasmic reticulum, whereas the active site of P450, also microsomal enzymes, the cytosolic site. UDPGA must be transported into the ER and the resulting glucuronide products generally need to be transported out to the ER into the cytosol (Zhang et al., 2007). There are more than 50 known microsomal membrane-bound isoenzyme in humans, however the major site of glucuronidation is the liver.

The types of glucuronides formed divide from the functional groups that conjugate with glucuronic acid. *O*-Glucuronides can be formed from phenols, alcohols, and carboxylic acids. Carboxylic acids are forming 'ester' glucuronides and

the other 'ether' glucuronides e.g. glucuronidation of morphine and salicylic acid. *O*-glucuronides are often excreted in bile and thus released into the gut where they can be broken down to the parent compound by *β*-glucuronidase and possibly reabsorbed. *N*-glucuronidation can be formed from amines (mainly aromatic), amides and sulfonamides. It has also been suggested that tertiary amines can form glucuronide giving quaternary nitrogen conjugates. *N*-glucuronides may form spontaneously, i.e. without the presence of the enzymes (Coleman et al., 2005). *S*-Glucuronidation, thiol groups can react with the UDPGA in the presence of UDPGT to yield *S*-glucuronides, e.g. antabuse glucuronidation. Direct attachment of glucuronic acid to the carbon skeleton of drugs (e.g. *C*-glucuronidation) has also been reported (Gibson and Skett, 2001).

The main families of UDPGT enzymes are divided into two families: UDPGT1, UDPGT2A and UDPGT2B. UDPGT1 families are a various forms that catalyze conjugation of planar phenols, bulky phenols, amines, tertiary amines and bilirubin. Nine active human forms now cloned are expressed, e.g. UDPGT1A1, UDPGT1A3-1A10. UDPGT2A family is olfactory UDPGT. UDPGT2B catalyze xenobiotics, steroids and bile acids e.g. UDPGT2B4, UDPGT2B7, UDPGT2B10, UDPGT2B15 and UDPGT2B17. Table 5.1 and Table 5.2 define the two major families and tissue distribution. Table 6.1 and Table 6.2 contain information on drug and other xenobiotic substrates, inhibitors and inducers (Zhang et al., 2007).

Table 5.1 UDPGT1A (Zhang et al., 2007)

Subfamilies	Tissue expression
UDPGT1A1	Liver, intestine, mammary gland
UDPGT1A2	-
UDPGT1A3	Liver, testes, prostate
UDPGT1A4	Liver, intestine
UDPGT1A6	Liver, kidney, intestine, brain, ovary, testes, spleen, skin
UDPGT1A7	Gastric epithelium esophagus
UDPGT1A8	Intestine, esophagus
UDPGT1A9	Liver, kidney, ovary, testes, spleen, skin, esophagus
UDPGT1A10	Intestine, lung
UDPGT1A11-13	-

Table 5.2 UDPGT2B (Zhang et al., 2007)

Subfamilies	Tissue expression
UDPGT2B1	Liver, low in kidney, intestine, testes
UDPGT2B2	Liver
UDPGT2B3	-
UDPGT2B4	Liver
UDPGT2B5	-
UDPGT2B6	-
UDPGT2B7	Liver, kidney, esophagus, intestine, brain (cerebellum)
UDPGT2B8	Liver
UDPGT2B9	Liver
UDPGT2B10	Liver, adrenals, prostate
UDPGT2Bi1	RNA present in liver, kidney, mammary gland, prostate, skin, adipose, adrenal, lung
DPGT2B12	Liver, kidney, intestine
UDPGT2B13	Adult liver
UDPGT2B14	Adult liver
UDPGT2B15	Liver, prostate, testes, esophagus
UDPGT2B16	-
UDPGT2B17	Liver, prostate, testes, uterus, skin, placenta, mammary gland, adrenals
UDPGT2B18	-
UDPGT2B19	-
UDPGT2B20	-
UDPGT2B21	-

Table 6.1 Selective substrates for individual UDPGT1A isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
UDPGT1A1	Bilirubin, estradiol (3-hydroxy), 2-hydroxyestrone, 2-hydroxyestradiol, trans-retinoic acid, catechol estrogens (2- and 4-hydroxy)	Ethinyl estradiol, morphine (3-hydroxy), buprenorphine, ferulic acid, genistein, naltrexone (low), naloxone (low), SN-38 (active metabolite of irinotecan), alizarin, quinalizarin	Bilirubin, chlorophenoxypropionic acid, chrysin, clofibrate, 3-methylchlolanthrene, phenylpropionic acid, phenobarbital, pregnenolone-16-nitrile and dexamethasone, clotrimazole, rifampin, St. John's wort	Atazenavir
UDPGT1A2	-	-	-	-
UDPGT1A3	Bile acid (carboxyl functional group), catechol estrogens (2-OH>4-OH), 2-OH-estrone 2-hydroxyestradiol, decanoic acid, dodecanoic acid, bilirubin (low)	Cyproheptadine, alizarin, buprenorphine, norbuprenorphine, bropirimine, diphenylamine, diprenophine, emodin, esculetin, eugenol, ezetimibe, fisetin, genestin, 3-hydroxydesloratadine, 7-hydroxyflavone, hydromorphine,	β-Naphthoflavone	-

Table 6.1 (con't) Selective substrates for individual UDPGT1A isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
		4-methylumbelliferone, morphine, nalorphine, naloxine, naltrexone, naringenin, quercetin, scopoletin, umbelliferone, *Carboxyl group*; clofibrate, ciprofibrate, etodolac, fenoprofen, ibuprofen, ketotifen, naproxen (racemic > *S*), valproic acid, formation of simvastatin and atorvastatin lactones via an intermediate acyl gluronide		
UDPGT1A4	*Estrogens*: 2-hydroxy-estrone and 2-hydroxy estradiol, estriol, *Progestins*: epiandrosterone, etiocholanone *Androgens*: dehydroepiandrosterone, testostereone	*Tritiary amines* : amitriptyline, chlorpromazine, clozapine, cyproheptadine, clozapine, diphenylamine, doxepin, imipramine, (*R*)-ketotifen, loxapine, promethazine, tripellenamine, trifluroperazene *Aromatic heterocyclic amines*: croconazole, lamotrigine, nicotine, 1-phenylimidazole, posaconazole, retigabine *Primary and secondary amines*: 2- and 4-aminobiphenyl, diphenylamine, desmethylclozapine *Alcoholic and phenolic substrates*: borneol, carveol, carvacrol, diogenin, hecogenin, isomenthol, menthol,	Phenobarbital, phenyltoin, carbamazepine	-

Table 6.1 (con't) Selective substrates for individual UDPGT1A isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
		neomemthol, 1- and 2-naphthol (low), *p*-nitrophenol (low), nopol, tigogenin		
UDPGT1A6	Serotonin, 3-hydroxymethyl DOPA	*Phenol:* acetaminophen, 2-amino-5-nitro-4-trifluoromethylphenol (flutamide metabolite), BHA, BHT, 7-hydroxy-coumarin, 4-hydroxycoumarin (low), dobutamine, 4-ethylphenol, 3-ethylphenol, 4-fluorocatechol, 2-hydroxybiphenyl, 4-iodophenol, 4-isopropylphenol (low), *Amines ::* 4-aminobiphenyl, 1-naphthylamine > 2-naphthylamine, N-OH-2-naphthylamine *Drugs :* acetaminophen, beta blocking adrenergic agents (low activity) such as atenolol, labetolol, propranolol, naproxen, salicylate, valproic acid	TCDD, β-naphthoflavone, 3-methylchloranthrene	α-Napthol, 4-butylphenol, 4-methylumbelliferone, 7-hydroxycoumarin

Table 6.1 (con't) Selective substrates for individual UDPGT1A isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
		Flavonoids: chrysin, 7-hydroxy flavone, naringenin		
UDPGT1A7	Estriol, 2-OH-estriol, 4-OH-estriol	Benzo(*a*)pyrene phenols (7-OH>>9-OH>-OH), benzo(*a*)pyrene-t-7, 8-dihydrodiol (7R-glucuronide, low affinity), 2-OH-biphenyl, 4-methylumbelliferone, 1- and 2-napthol, 4-nitrophenol, octylgallate, vanillin	TCDD	-
UDPGT1A8/9*	2-hydroxyestrone, 4-hydroxy estrone, 2-hydroxy estradiol, 4-hydroxy estradiol, estrone, dihydrotestosterone, trans-retinoic acid, 4-hydroxy retinoic acid, hyodeoxycholic, testosterone, leukotriene B4	Alizarin, anthraflavic acid, apegenin, emodin, fistin, flavoperidol, genistein, naringenin, quercetin, quinalization, 4-methylumbelliferone, scopoletin, carvacrol, eugenol, 1-naphthol, *p*-nitrophenol, 4-aminobiphenyl, 2-hydroxy, 3-hydroxy, and 4-hydroxy biphenyl, buprenorphine (low), morphine (low), naloxone, naltrexone, ciprofibrate, difunisal, diphenylamine, furosemide, mycophenolic acid (high), phenolphthalein, propofol, valproic acid, nandrolone, (-)-epigallocatechin gallate (tea phenol), trogitazone, raloxifene, quercetin, luteolin	3-Methyl cloranthrene	-

Table 6.1 (con't) Selective substrates for individual UDPGT1A isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
UDPGT1A9/8*	Retonoic acid, thyroxine (T4), tri-iodothyronine (T3 ; minor), 4-hydroxyestrone, 4-hydroxyestradiol (major)	*Planar phenol:* phenol, acetaminophen, 2-hydroxybiphenyl, 4-iodophenol, 4-propylphenol *Bulky phenol:* phenol red, flurescein, phenolphthalein *Simple catechols:* octyl gallate, propyl gallate *Primary amines*: 4-aminobiphenyl *Xenobiotics:* acetaminophen, gemfibrozil, atenolol, labetalol, propranolol, diflunisal, fenoprofen, metoprolol, pindolol, troglitazone, clofibric acid	TCDD, tetrabutyl hydroquinone, clofibric acid	High concentration of propofol
UDPGT1A10	2-OH-estrone (low), 4-OH estrone (low), dihydrotestosterone, testosterone	Alizarin, anthraflavic acid, emodin, fisetin, genistein, naringenin, quercetin, quinalizarin, scopoletin, carvacrol, eugenol, mycophenolic acid, nandralone, 1-methyl-5-α-androst-1-en-17β-ol-3-one (metabolite of metenolone), 5α-androstane-3α,17β-diol (metabolite of testosterone), SN-38 (minor), raloxifen	-	-
UDPGT1A11	-	-	-	-
UDPGT1A12	-	-	-	-

Table 6.2 Selective substrates for individual UDPGT2B isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
UDPGT2B1	Estradiol (17-hydroxy)	Morphine	-	-
UDPGT2B2	-	-	-	-
UDPGT2B3	Bile acid	-	-	-
UDPGT2B4	Metabolites of polyunsaturated fatty acids (PUFA)	*Phenols:* eugenol, 4-nitrophenol, 2-aminophenol, 4-methyl umbelliferone, morphine	Fenofibric acid, chenodeoxycholic acid-activated FXR	-
UDPGT2B5	-	-	-	-
UDPGT2B6	-	-	-	-
UDPGT2B7	Arachidonic acid metabolites: leukotriene B4 (LTB4) bile acid, estrogens, pregnanes, androgens	*R*-Oxazepam, naproxene, menthol, zidovudine, abacavir, acetaminophen, almokalant, carvedilol, chloramphenicol, epirubicin, 1′-hydroxy estragole, 5-hydroxy rofecoxib, lorazepam, menthol, 4-methylumbelliferone, 1-naphthol (low), 4-nitrophenol, octylgallate, propranolol,	Rifampicin, phenobarbital, HNFHNF1α	*R*-Oxapam, zidovudine, flunitrazepam relatively potent, doclofenac, etonitazenyl

Table 6.2 (con't) Selective substrates for individual UDPGT2B isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
		tamazepam, maxipost *Caboxylic acid-containing drugs:* benoxaprofen, ciprofibrate, clofibric acid, diflunisal, dimethylxanthenone-4-acetic acid (DMXAA), fenoprofen, ibuprofen, indomethacin, ketoprofen, naproxen, pitavastation, simvastatin acid, tiaprofenic acid, valproic acid, zaltoprofen, zomepirac, naproxen *Opioids:* morphine 3OH>6OH, buprenorphine, nalorphine, naltrexone, codeine (low), naloxone		
UDPGT2B8	-	-	-	-
UDPGT2B9	C18, C19, C20 steroids, fatty acids (C6-C12)	Morphine	-	-
UDPGT2B10	-	-	-	-
UDPGT2B11	-	-	-	-

Table 6.2 (con't) Selective substrates for individual UDPGT2B isozymes (Zhang et al., 2007)

Isoenzyme	Endogenous substrates	Drug or xenobiotic substrates	Inducers	Inhibitors
UDPGT2B12	-	-	-	-
UDPGT2B13	17β-estradiol	4-hydroxy biphenyl	-	4-hydroxy biphenyl
UDPGT2B14	Estriol	-	-	-
UDPGT2B15	Testosterone, dihydrotestosterone	-	-	-
UDPGT2B16	-	-	-	-
UDPGT2B17	Testosterone, androsterone	-	-	-
UDPGT2B18	-	-	-	-
UDPGT2B19	-	-	-	-
UDPGT2B20	-	-	-	-
UDPGT2B21	-	-	-	-
UDPGT2B28	-	17β-estradiol, testosterone	-	-

Sulfation

Sulfation of chemicals involves the conjugation of the substrate with a sulfonyl (SO_3). The co-factor PAPS (3′-phosphoadrenosine-5′-phosphosulphate) acts as the sulfonyl donor and the reaction is catalyzed by sulfotransferase (SULT) enzyme. Conjugation can occur at -C-OH, -N-OH and -NH side chain to yield *O*-sulfates and *N*-sulfates. PAPS synthesis from inorganic sulfate (SO^{2-}_4), and ATP by enzymes sulfurylase and adenosine-5′-phosphosulfate kinase (APS-kinase) in prokaryotes, and a bifunctional enzyme PAPS synthetase (PAPSS) in higher organisms including humans. The reaction of sulfation as shown in Figure 3:

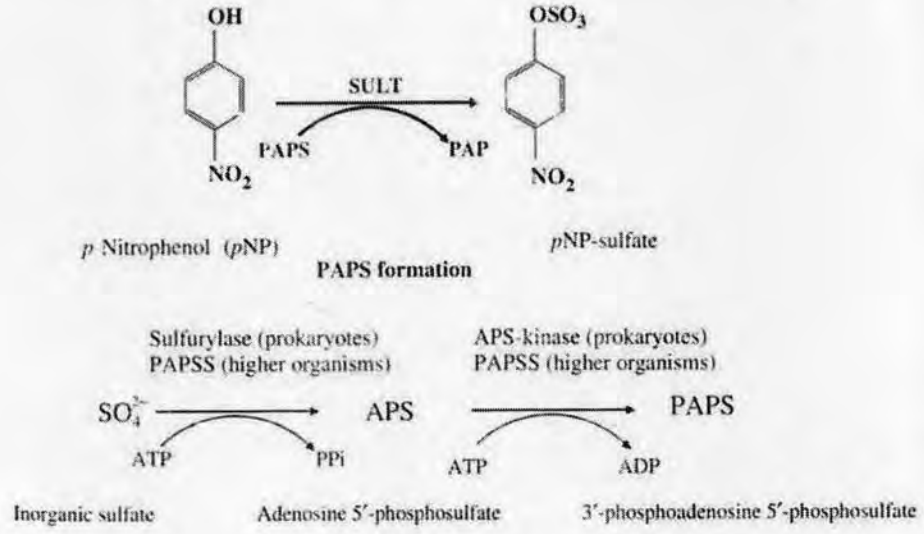


Figure 3 The overall sulfation reaction, and formation of the co-factor PAPS (Zhang et al., 2007).

Sulfation is generally a detoxification pathway where the conjugated product has greater water solubility and is therefore excreted more readily from the body mainly in urine. Some chemicals have been shown to form mutagenic and carcinogenic reactive electrophiles upon sulfation such as minoxidil and cholecystokinin.

The cytosolic SULT exist as homodimer or heterodimers in solution. Membrance-bound SULTs are present in the golgi apparatus of cell and catalyze sulfation and posttranslational modification of peptides, proteins, lipids and glycosaminoglycans. SULT activity found to be the highest in the liver and small intestine, and also high in adrenal tissue, brain, kidneys (Zhang et al., 2007).

Various forms of this enzyme prefer their substrates and classifies by molecular and gene structure. Table 7 show SULT isoforms and their substrates.

Table 7 SULT and their substrates (Gibson and Skett, 2001)

Isozyme	Substrates	Sites
Phenol sulfotransferase	Isoprenaline	Liver, kidney, gut
Alcohol sulfotransferase	Dimetranidazole	Liver
Steroid sulfotransferase	Oestrone	Liver
Arylamine sulfotransferase	Paracetamol	Liver

The cytosolic SULTs form is a large superfamily of genes. Members of the supperfamily are assigned families and subfamilies based on amino acid homology. The family member share at 45% amino acid sequence identify, while subfamily member share at 60% amino acid sequence. To date, 45 SULTs have been identified in mammals and 11 SULTs isoforms have been discovered in human. These are listed in Table 8 with their substrates. Several drugs and xenobiotics are known to induce or inhibit SULT, as shown in Table 9.

Table 8 Human SULT isoforms and their substrates (Gibson and Skett, 2001; Zhang et al., 2007)

Human SULT cDNA	Substartes
SULT1A1	Simple phenol (i.e. *p*-nitrophenol and α-naphthol), 17β-estradiol, iodothyronines, acetaminophen, minoxidil, 17α-ethinylestradiol, isoflavones, hydroxyl-tamoxifen
SULT1A2	Catecholestrogens, simple phenol (i.e. *p*-nitrophenol and α-naphthol)
SULT1A3	Dopamine, tyramine, serotonin, salbutamol, isoprenaline, dobutamine, hydroxylated tibilone, 4-hydroxypropranolol
SULT1B1	Simple phenol (i.e. *p*-nitrophenol and α-naphthol), catechols, iodothyronines, *0*-desmethylnaproxen
SULT1C2	*N*-hydroxy-2-acetylaminfluorene
SULT1C4	*N*-hydroxy-2-acetylaminfluorene
SULT1E1	Estrone, 17β-estradiol, 17α-ethinylestradiol, equilenin, diethylstilbestrol, thyronine, *0*-desmethylnaproxen, 3-OH-benzo[*a*]pyrene, phytoestrogens

Table 8 (con't) Human SULT isoforms and their substrates (Zhang et al., 2007)

Human SULT cDNA	Substartes
SULT2A1	DHEA, pregnolone, cholesterol, cortisol, testosterone, bile salts, PAHs (benzylic alcohols), hydroxyl-tamoxifen
SULT2B1_v1	DHEA, pregnolone, 3β-hydroxy steroids
SULT2B1_v2	DHEA, pregnolone, 3β-hydroxy steroids
SULT4A1	?

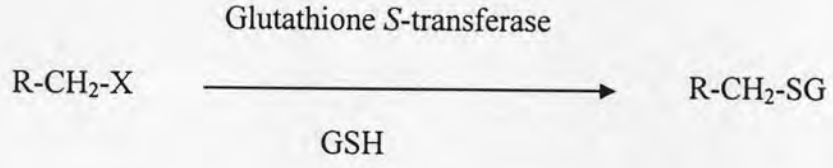
Table 9 Substrates, inducers and inhibitors of SULT (Allera et al., 2004; James et al., 2008; Wang et al., 2006; Zhang et al., 2007)

Enzyme	Substrates	Inhibitors (for all SULT)	Inducers (for all SULT)
SULT	3,3′-di-iodothyronin, 17β-estradiol, 2-naphthol, 4-phenylphenol, tyramine, 4-methylphenol, catechol, 4-isopropylphenol, minoxidil, paracetamol, iodothyronines, hydroxyarylamines, 5-hydroxytryptamine, salbutamol, isoprenaline, acetylaminofluorene, estrone, estradiol, estriol, 17α-ethinyl estradiol, equilenin, thyroxine, diethylstilbestrol, DHEA, pregnenolone, cholesterol, cortisol, benzyl alcohols of PAHs, testosterone, bile aminophenols, tyrosine methyl ester, epinephrine, ethanol, tyrosine residues, quercetin, hydroxysteroids, curcuminoids, genistein	polychlorobiphenylols, 4-chlorobiphenyl-3′-ol dichloronitrophenol, triphenyltin, fenarimol, prochloraz, methyltestosterone, clomiphene, diuron, salicylic acid, thiol reagens, 2,6-dichloro-4-nitrophenol, pentachlorophenol, diadzein, baicalcin, mefenamic acid, naproxen, salicylic acid, quercetin, galangin, kaempferol, fisetin, myricetin, chrysin, apigenin, genistein, curcumin, ellagic acid	progesterone, medrogestrone, promegestone or tibolone, 4-OH-tamoxifen, retinoic acid, endogenous and exogenous estrone, triamcinolone, acetonide, farnesol

Glutathione conjugation

Glutathione (GSH) is recognized as a protective role within the body in removal of potentially toxic elctrophilic compounds such as phase I metabolites of many drugs, epoxides, haloalkanes, nitroalkanes, alkenes and aromatic halo- and nitrocompounds. These elctrophilic compounds can react with glutathione to form non-toxic conjugates. GSH is present at highest concentration in the liver, with higher values in the cytosol, and also in mitochondria and nucleus. The glutathione conjugates may be excreted directly in urine, or more usually in bile, but more commonly undergo further metabolism. The tripeptide glutathione (Gly-Cys-Glu), once attached to the acceptor molecule, can be attacked by γ-glutamyltranspeptidase, which removes the glutamate, and a peptidase, with removes the glycine, to yield the cysteine conjugate. These two enzymes are found in the liver and kidney cytosol.

The glutathione *S*-transferase (GST) enzymes are proteins which catalyzes the conjugation of a variety of compounds with the endogenous tripeptide glutathione as following:



where R-CH_2-X represents literally hundreds of electrophillic drug and xenobiotic such as epoxides, haloalkanes, nitroalkenes, and R-CH_2-SG represent the glutathione adduct (Gibson and Skett, 2001). Almost all the GSTs are found in the cytosol, although some are associated with the endoplasmic reticulum. The hydrophobic GSTs in endoplasmic reticulum are structurally different from the cytosolic GSTs and they metabolized leukotrienes and prostaglandins (Coleman et al., 2005).

Over 20 isoforms of GSTs are known to expressed in man. The nomenclature system has been confusing, a unifying nomenclature for the soluble human transferases has been suggested and classified into gene family: Alpha (A), Mu (M), Pi (P), Sigma (S), Theta (T), Zeta, Omega and Kappa and reflect their subunit composits. Thus GSTA1-1 is a GST of the Alpha class, consisting of homodimer of two identical '1-type sub-units', which found in only a few tissues of the body, including kidneys, intestine, lung, liver and testis. The GST has broad substrates

specificity, as shown in Table 10. Substrates, inducers and inhibitors of GST are shown in Table 11.

Table 10 Specific substrates of GST (Coleman et al., 2005; Zhang et al., 2007)

Enzyme	Specific substrates
GST Alpha	Δ^5-androstene-3,17-dione (isomerase), Δ^5-pregnane 3,20-dione, busulfan, chlorodinitrobenzene (CDNB) (moderate), 1,2-dichloro-4-nitrobenzene (DCNB) (low), various nitrogen mustard such as chlorambucil and melphalan, doxorubicin, vincristine, etoposide, mitoxantrone, fatty acid hydroperoxides
GST Mu	*trans*-4-phenyl-3-buten-2-one, ethacrynic acid, Δ^3-androstene-3, 17-dione, chlorodinitrobenzene (CDNB) (moderate), 1,2-dichloro-4-nitrobenzene (DCNB) (high), aflatoxin B1-*exo* 8,9-epoxide, *p*-nitrophenyl acetate, styrene 7,8-epoxide, cumene hydroperoxide, ethacrynic acid, 2-cyano-1,3-dimethyl-lnitroso-guanidine
GST Pi	1-chloro-2,4-dinitrobenzene (CDNB) (moderate), acrolein, adenine, propenal, benzyl isothiocyanate, 4-vinylpyridine, atrazine, ethacrynic acid, 7-nitro-2,1,3-benzoxadiazole, trichloroethylene, COMC-6, 4-oxo-nonenal,
GST Theta	polycyclic aromatic hydrocarbons, halomethanes, dihalomethanes, ethylene oxide, 1,1-dichloropropene, phenethylisothiocyanate
GST Omega	1-chloro-2,4-dinitrobenzene (CDNB), 7-chloro-4-nitrobenzo-oxa-1,3-diazole, *p*-nitrophenol acetate, *S*-(4-nitrophenacy)glutathione
GST Zeta	dichloroacetic acid and other haloacetic acid, maleylacetoacetate *cis-trans* isomerase, ethacrynic acid (low)

Table 11 Substrates, inducers and inhibitors of GST (Baijail et al., 1997; Schultz et al., 1997; Turella et al., 2005; Wallin et al., 1988)

Enzyme	Substrates	Inhibitors (for all GST)	Inducers (for all GST)
GST	estradiol, cortisol, testosterone, urethane, paracetamol, parathion, tetracycline, penicillin, ethacrynic acid, 17β-estradiol, sulfobromophthalein, *p*-nitrobenzylchloride (NBC), vitamin K3, 1,2-epoxy-3-(*p*-nitrophenoxy)propane, hexachloro-1,3-butadiene nitrobenzene	equine estrogens, sulfobromophthalein, ethanol, clofibate, ethacrynic acid, GSH analogs, gossypol, indomethacin, misonidazole, piriprost, sulfasalazine, terrapin199, selected 7-nitro-2,1,3-benzoxadiazole, quercetin	17β-estradiol, 4-nonylphenol, butyrated, hydroxyanisole, ethoxyequin, oltipraz, cumarin, indole-3-carbinol, TCDD, 3-methylcholanthrene, folic acid, polycyclic aromatic hydrocarbons, *trans*-stilbene oxide, nitrogen mustard alkylating agent (i.e. melphalan, bromophenol), alkyl sulfonates, hypolipidemic drugs, barbiturates, Phenobarbital, carnesol, styrene, procarbazine, polychlorinated biphenyls, thiotepa, mitomycin C

NAD(P)H quinone oxidoreductase (NQOR)

NAD(P)H quinone oxidoreductase (NQOR), is a cytosolic flavoprotein also know as DT diaphorase that catalyzes the two-electron reduction of quinone or quinoid compounds to hydroquinones (both *para* and *ortho*), using NADH or NAD(P)H as electron donor.

NQOR catalyzes the reduction of quinones to hydroquinones as following:

$$Q + NAD(P)H + H^+ \longrightarrow QH_2 + NAD(P)^+$$

where Q is a quinone, it also reacts with a number of other electron acceptors, for example, various azo dyes and nitro compounds. The most NQOR reactions are two electron reductions, avoiding the generation of radicals, unlike the one electron reduction of quinines by NADPH cytochrome P450 reductase. However, the product hydroquinones may react with O_2 to generate O^-_2 (Zhang et al., 2007).

NQOR is widely distributed in the animal tissues. Liver is one of the richest sources of the enzyme, but other tissues, including brain, heart, lung, kidney, small intestine, skeletal muscle, and mammary gland, exhibit varying NQOR activities. On fractionation of liver homogenates, the bulk (>90%) of NQOR is recovered in the cytosolic fraction, whereas minor portions of the enzyme associated with mitochondria and microsomes (Ernster, 1967; 1990).

NQOR is a dimmer of two equal subunit each containing FAD. Mouse, rat and human appear to process two, three and four of NQOR, respectively. The human enzymes are encoded by four distinct gene loci (NQO1, NQO2, NQO3 and NQO4). The major NQOR is coded for by the NQO1 gene but roles of the other three are poorly understood. NQO1 (NAD(P)H: quinone acceptor oxidoreductase) is a flavoprotein that catalyzes the two-electron reduction of quinones or quinoid compounds to hydroquinones, using either NADH or NADPH as the electron donor. NQO1 catalyzes a two-electron reduction of quinones, without the formation of semiquinones. It consists of two identical subunits. Each subunit has a molecular weight of 30,000 and contains one flavin adenine dinucleotide (FAD) prosthetic group, noncovalently attached to the protein.

NQO1 can function physiologically as one of several vitamin K reductases in the vitamin K cycling involved in the hepatic posttranslational modification of vitamin K hydroquinone– dependent blood coagulation factors. NQO1 is also known to reductively activate cytotoxic anti-tumor quinones such as mitomycins, anthracyclines, and aziridinyl benzoquinones, as well as nitrobenzamides such as 5-(aziridin-1-yl)-2,4-dinitro-benzamide.

NQO2 (dihydronicotinamide riboside: quinone oxidoreductase) has a high nucleotides sequence identity to DT-diaphorase and is considered to be an isozyme of

DT diaphorase. The function of NQO2 is not clearly understood. NQO2 was found to be expressed selectively in kidney, skeletal muscles, liver, heart, and lung, suggesting a tissue-specific action of the enzyme. The enzyme catalyzes the oxidation of dinitronicotinamide riboside-dependent oxidoreductase, an unusual electron donor. NQO2 reduces vitamin K, such as menadione. It may function physiologically as one of several vitamin K reductases in the vitamin K cycling involved in the hepatic posttranslational modification of vitamin K hydroquinone-dependent blood coagulation factors. NQO2 would be have as a dicumarol-insensitive vitamin K reductase (Chen et al., 2000).

Specific substrates, inducers and inhibitors of NQOR are shown in Table 12.

Table 12 Specific substrates, inducers and inhibitors of NQOR (Benson et al., 1980; Ernster et al., 1960; James et al., 2008, Moon et al., 2006)

Enzyme	Specific substrates	Inhibitors (for all NQOR)	Inducers (for all NQOR)
NQOR	menadione, vitamin K_1, benzo(*a*)pyrene-3,6-quinone, cyclized-dopamine ortho-quinone, biotinylalted lectins, *N*-acetylgalacto-samine, mannose, galactosly(*β*-1,3)-*N*-acetylgalactosamine	clofibrate, sulfhydryl reagents (*p*-chloromeribenzoate, *O*-iodosobenzoate), thyronine (thyroxine, 3,3′,5-triiodothyronine, tetraiodothyrono-acetate), flavins, flavin antagonists (FAD, FMN, atabrine, chlorpromazine)	estradiol, farnesol, diallyl sulfide, phenolic antioxidants, 1,2-dithiole-3-thiones, isothiocyanates, sudan III [1-[[4 phenylazo) phenyl[azo]-2 naphthalenol], 3-methylcholanthrene, serum albumin, neutral phospholipids, detergents, flavanone, naringenin, xanthohumol, dehydrocycloxantholhumol hydrate, morin, flavonoid pinostrobib, silymarin, genistein, daidzein, flavone, coumarins