ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cytochrome P450s in Breast Cancer.

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ABSTRACT

The cytochrome P450s (P450s) are key drug metabolizing enzymes in the human body , Drug metabolism via the cytochrome P450 system has emerged as an important determinant in the occurrence of several drug-drug interactions. A greater degree of interaction predictability has been achieved through the identification of P450 isozymes and some of the drugs that share them Tamoxifen (TAM) is an important anticancer drug that is commonly used in the prevention and treatment of breast cancer, and also exhibits antioxidant and cardioprotective effects. One mechanism by which TAM inhibits cancer cell growth is competitive blocking of estrogen receptors. However, TAM also inhibits the growth of estrogen-receptor-negative breast cancer cells. This implies the presence of additional mechanisms that are not related to estrogen receptor mediation. TAM was also found to antagonize protein kinase C without interacting with the enzyme's active site.

Keywords: Cytochrome P450 (CYPs), DRUG METABOLISM IN CYP2D6, Breast cancer, Tamoxifen, Metabolism.

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ISSN: 0975-8585

Cytochrome P_{450} (CYPs):

Cytochrome P450s (CYPs), constituting a superfamily of heme-containing monooxygenases found in all three domains of life, are involved in the metabolism of a diverse array of endogenous (e.g. steroids and lipids) and exogenous (i.e. xenobiotic compounds) [1]. Cytochrome P450 enzymes are essential for the metabolism of many medications. Althoughthis class has more than 50 enzymes, six of them metabolize 90 percent of drugs, with the twomost significant enzymes being CYP3A4 and CYP2D6 [2]. generally have more flexible active sites to allow them to act on a wider array of substrates [3]. The name cytochrome P450 derives from the fact that the CO bound heme complex has an absorption band at 450 nm. Their heme active site is linked to the protein via a thiolate linkage of a cysteinate residue covalently bound to the iron Centre [4]. CYP450s are hemoproteins and act as the terminal oxidases in the monooxygenase system [5]. The three components of the P450 monooxygenase system are P450, which acts as the substrate binding protein, NADPH-cytochrome P450 reductase (CPR), which transfers electrons from NADPH to CYPs, and cytochrome b5, which transfers electrons from NADH to CYPs in some P450 monooxygenase systems as an additional potential electron donor [6].

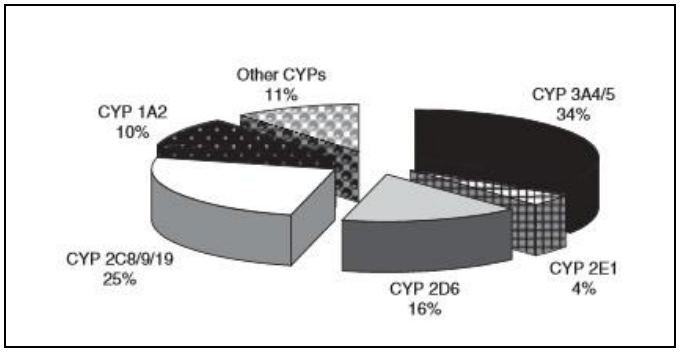


Figure (1): Proportion drugs metabolized by CYP450 isoforms[7].

CYP $_{450}$ CATALYTIC MECHANISMS:

The principal features of the consensus mechanism of cytochromeP450 are as outlined in Figure1 , The oxidation chemistry occurs in steps 7 and 8 [8]:

(1) binding of substrate to the enzyme, sometimes accompanied by a spin state change of the iron, to afford an enzyme-substrate adduct 3;
(2) reduction of the ferric cytochrome P450 by an associated reductase with an NADPH-derived electron to the ferrous cytochrome P450 4;
(3) binding of molecular oxygen to the ferrous heme to produce a ferrous cytochrome P450-dioxygen complex 5, similar to the situation in oxymyoglobin;
(4) a second one-electron reduction and protonation to arrive at the Fe(III)- hydroperoxy complex 6;
(5) protonation and heterolytic cleavage of the O-O bond in 6 with concurrent production of a water molecule to form a reactive iron-oxo intermediate 7;
(6) and, finally, oxygen-atom transfer from this iron-oxo complex 7 to the bound substrate to form the oxygenated produc complex 8. Product dissociation completes the cycle [9].

ISSN: 0975-8585

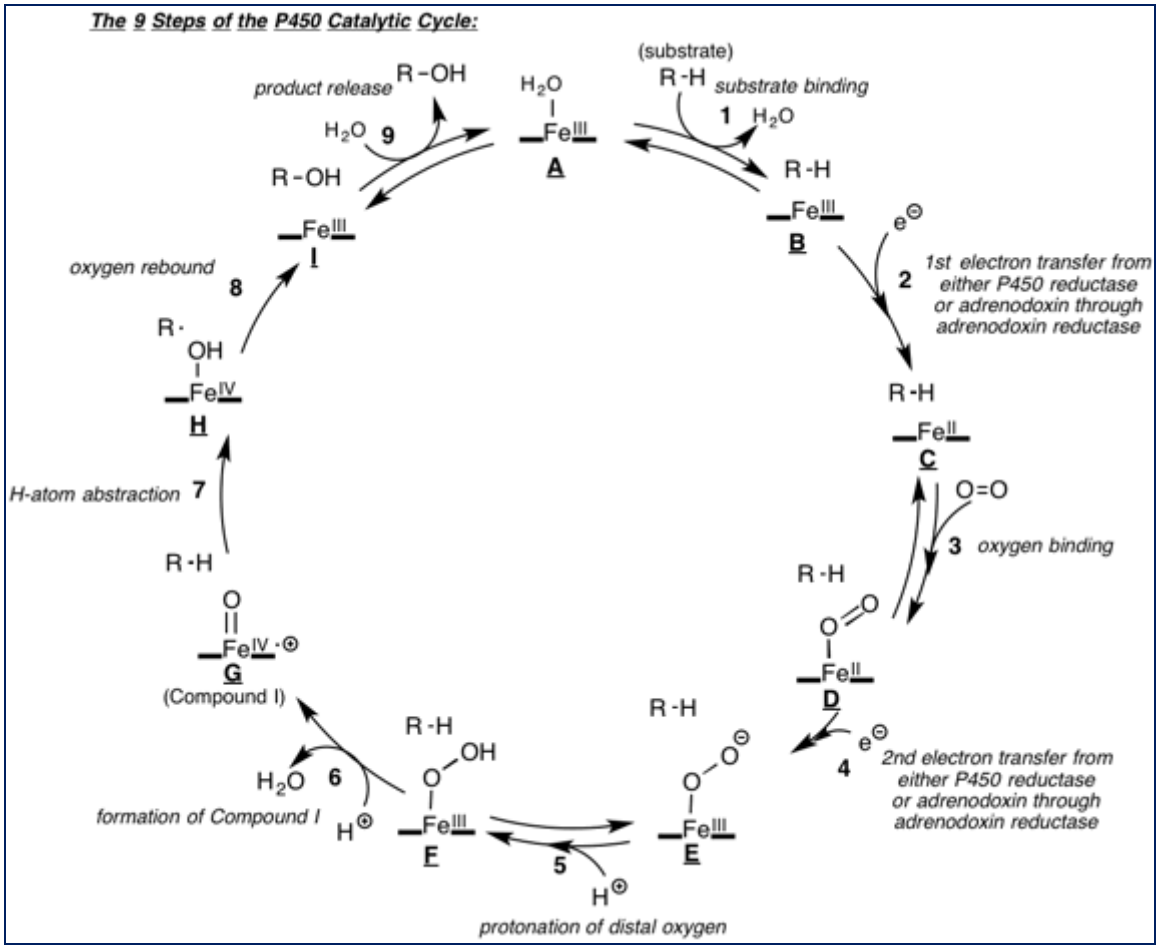


Figure (2): Generalized catalytic cycle for P450 reactions[10].

Cytochrome P450 drug metabolism:

The cytochrome P450s (P450s) are key drug metabolizing enzymes in the human body , Drug metabolism via the cytochrome P450 system has emerged as an important determinant in the occurrence of several drug-drug interactions. A greater degree of interaction predictability has been achieved through the identification of P450 isozymes and some of the drugs that share them. Six different P450 isozymes—CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP2E1, and CYP3A4—that play important roles in drug metabolism have been identified [11].The cytochrome P450 family of heme monooxygenase enzymes (P450s) plays an important role in the metabolism of drugs. Potentially harmful complications can sometimes occur during P450-mediated metabolism, such as drug–drug interactions and formation of toxic metabolites. Most drug molecules contain several sites that are susceptible to P450-mediated oxidation, and the site of oxidation may determine whether a toxic metabolite is formed [12]..

Mechanism of CYP inhibition:

The inhibition of CYP substrate metabolism occurs during substrate binding specifically during the binding of molecular oxygen to the ferrous (Fe2+) atom in the substrate. The transfer of activated oxygen from the heme iron in the substrate is positioned at a vulnerable phase in the inhibition cascade . This inhibition of metabolism of one drug by another drug may cause considerable elevations in the exposure of one or both drugs which puts the patient at risk to serious adverse effects. However, CYP inhibitors may cause a decrease in the concentrations of the active metabolite when interacting with prodrugs, such as clopidogrel, causing an inactivation of the drug . The onset of inhibition usually occurs relatively fast compared to induction. Generally, CYP inhibition is divided into reversible, quasi-irreversible and irreversible interactions. The nature of the substrate binding to the iron of the heme is a complex mechanism .

ISSN: 0975-8585

Mechanism of CYP induction:

The human body has the ability to reduce environmental xenobiotic pressure by accelerating the function of specific drug-metabolizing enzymes. IncreasedCYP enzyme synthesis is mediated by ligand-activated transcription factorsand receptors such as the aryl hydrocarbon receptor (AhR), pregnane Xreceptor (PXR) and constitutive androstane receptor . Inaddition to enhanced transcription of the CYP gene, the concentrations ofintracellular CYPs may be elevated by a decreased rate of protein degradation. In some cases, non-receptor-mediated induction processesmay be involved. Many receptors that mediate CYP enzyme transcription arepresently unknown . Maximal induction is a gradual process andthese levels may be reached after a few days to two weeks. Typical, inducibleCYP enzymes include CYP1A1, 1A2, 2A6, 2C9, 2C19, 2E1 and 3A4. Inhumans, the antimicrobial agent, rifampicin, is a potent enzyme inducers[13].

	P450 isozyme					
Function	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP2E1	CYP3A4
Substrates of isozyme:	caffeine clozapine cyclobenzaprine fluvoxamine imipramine mexiletine olanzapine pimozide propranolol tacrine theophylline warfarin	amitriptyline citalopam clomipramine cyclophosphamide diazepam imipramine lansoprazole nelfinavir omeprazole phenytoin	amitriptyline *(demethylation)* celecoxib diclofenac flurbiprofen ibuprofen losartan *(not candesartan)* naproxen phenytoin piroxicam sulfamethoxazole tolbutamide warfarin	amitriptyline clomipramine codeine desipramine dextromethorphan imipramine metoprolol nortriptyline oxycodone paroxetine propafenone risperidone thioridazine timolol tramadol venflaxine	acetaminophen chlorzoxazone dapsone enflurane ethanol halothane isoflurane isoniazid	alprazolam astemizole buspirone calcium channel blockers carbamazepine cisapride cyclosporine doxorubicin erythromycin etoposide felodipine fentanyl HIV protease inhibitors ifosphamide lovastatin *(not pravastatin)* midazolam nifedipine pimozide quinidine quinine simvastatin tacrolimus terfenadine triazolam
Inhibitors of isozyme:	cimetidine ciprofloxacin citalopram diltiazem enoxacin erythromycin fluvoxamine mexiletine ofloxacin tacrine ticlopidine	cimetidine felbamate fluoxetine fluvoxamine ketoconazole lansoprazole omeprazole paroxetine ticlopidine	amiodarone fluconazole fluoxetine fluvastatin isoniazid metronidazole paroxetine phenylbutazone sulfamethoxazole/ trimethoprim sulfaphenazole ticlopidine	amiodarone chlorpheniramine fluoxetine haloperidol indinavir paroxetine propafenone quinidine ritonavir sertraline thioridazine ticlopidine	disulfiram water cress	amiodarone cimetidine cyclosporine danazol diltiazem fluconazole *(large doses)* grapefruit juice HIV protease inhibitors itraconazole ketoconazole macrolides *(not azithromycin)* miconazole nefazadone omeprazole quinidine ritonavir verapamil
Inducers of isozyme:	carbamazepine tobacco	carbamazepine norethindrone *(not phenobarb.)*	phenobarbital rifampin secobarbital		chronic ethanol isoniazid tobacco	carbamazepine rifabutin rifampin ritonavir

*Table compiled from references 1, 2, 4, 8.

Figure(3): ytochrome P450 drug metabolism

ISSN: 0975-8585

Cytochrome P450 2D6

The human cytochrome P450 (CYP) 2D6 gene is a member of the CYP2D gene subfamily . Even though CYP2D6 only represents 1–5 % of the CYP liver content, it is responsible for the oxidative metabolism of up to 25 % of commonly prescribed drugs such as antidepressants, antipsychotics, opioids, antiarrythmics and tamoxifen, many of which have a narrow therapeutic window . CYP2D6 is encoded by a highly polymorphic gene, with more than 70 alleles and 130 genetic variations described [14]. The human CYP2D6 enzyme has a high affinity for alkaloids, and it detoxifies alkaloids [15].

VARIOUS ASPECTS OF CYP2D6:

Cytochrome P450 2D6 (CYP2D6), a member of the cytochrome P450 mixed-function oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the body. Its 3D structure is shown in Figure 4. Whilst CYP2D6 is involved in the oxidation of a wide range of substrates of all the CYPs, there is considerable variability in its expression in the liver. The gene is located near two cytochrome P450 pseudogenes on chromosome 22q13.1. Alternatively, spliced transcript variants encoding different isoforms have been found for this gene.

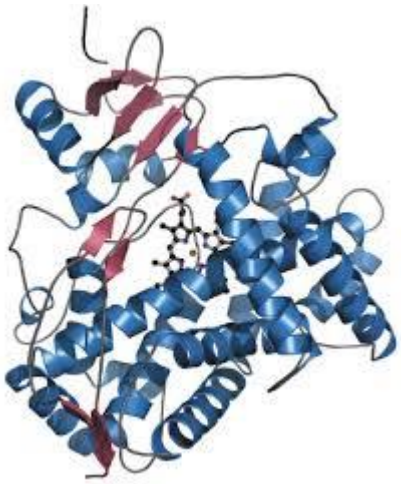


Figure (4): 3D Structure of CYP2D6.

CYP2D6 shows the largest phenotypical variability amongst the CYPs, largely due to genetic polymorphism. The genotype accounts for the normal, reduced and nonexistent CYP2D6 function in subjects. More than 50 human CYP Isoenzymes have been identified. The genetic basis for extensive and poor metaboliser variability is the CYP2D6 allele, located on chromosome 22. Subjects who possess certain allelic variants will show normal, decreased or no CYP2D6 function depending on the allele. In CYP2D6, genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism. Extensive metabolism (EM) of a drug is characteristic of the normal population; poor metabolism (PM) is associated with accumulation of specific drug substrates and is typically an autosomal recessive trait requiring mutation and/or deletion of both alleles for phenotypic expression; and ultra extensive metabolism (UEM) results in increased drug metabolism and is an autosomal dominant trait arising from gene manner in 5-10% of the Caucasian population and is now associated with the inefficient metabolism of over 30 drugs with a wide range of clinical indications [16].

DRUG METABOLISM IN CYP2D6:

Due to the high polymorphic character of CYP2D6, this enzyme is also the site of a number of drug interactions in vivo, which are of clinical significance [17].. The basic purpose of drug metabolism in the body is to make drugs more water soluble and thus more readily excreted in the urine or bile. One common way of metabolizing drugs involves the alteration of functional groups on the parent molecule (e.g., oxidation) that is, the cytochrome P450 enzymes e.g., CYP2D6. Drug interactions involving the cytochrome P450 isoforms generally result from one of two processes, enzyme inhibition or enzyme induction. Enzyme inhibition usually

involves competition with another drug for the enzyme binding site. This process usually begins with the first dose of the inhibitor, and onset and offset of inhibition correlate with the half-lives of the drugs involved. Enzyme induction occurs when a drug stimulates the synthesis of more enzyme protein, enhancing the enzyme's metabolizing capacity [18].

CYP2D6 phenotype–genotype relationships:

- **Poor metabolizers**: individuals with absent CYP2D6activity; poor metabolizers are carriers of twononfunctional alleles of CYP2D6, resulting innonfunctional CYP2D6 enzymes.

- **Extensive metabolizers**: individuals with normalactivity; most extensive metabolizers carry one ortwo alleles encoding normally functional CYP2D6activity.

- **Intermediate metabolizers**: individuals with reducedactivity; most intermediate metabolizers carry onenonfunctional allele and one intermediate metabolizerallele encoding an enzyme with subnormal CYP2D6activity.

- **Ultrarapid metabolizers**: subjects with increasedCYP2D6 activity; genetically determined ultrarapidmetabolizers carry at least one duplicated ormultiduplicated functional allele [19].

Breast cancer:

Breast cancer is one of the most common and serious malignancies worldwide. Despite intensive cancer control efforts, it remains the second-leading cause of cancer death among women [20]. MCF-7 is the acronym of Michigan Cancer Foundation -7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers [21]. MCF7 is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Cancers originating from ducts are known as ductal carcinomas, while those originating from lobules are known as lobular carcinomas. Breast cancer occurs in humans and other mammals. While the overwhelming majority of human cases occur in women, male breast cancer can also occur[22]. MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen, in the form of estradiol, via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line [23]

Drug Activated by CYP2D6:

Tamoxifen (Nolvadex)

Tamoxifen (TAM) is an important anticancer drug that is commonly used in the prevention and treatment of breast cancer , and also exhibits antioxidant and cardioprotective effects . One mechanism by which TAM inhibits cancer cell growth is competitive blocking of estrogen receptors . However, TAM also inhibits the growth of estrogen-receptor-negative breast cancer cells . This implies the presence of additional mechanisms that are not related to estrogen receptor mediation. TAM was also found to antagonize protein kinase C without interacting with the enzyme’s active site . Other studies have shown that TAM can cause liver toxicity by affecting mitochondria functions . Since TAM is highly lipophilic , modulation of cell membrane structural and mechanical properties may be a reason for its anticancer activity and associated adverse effects [24].

ISSN: 0975-8585

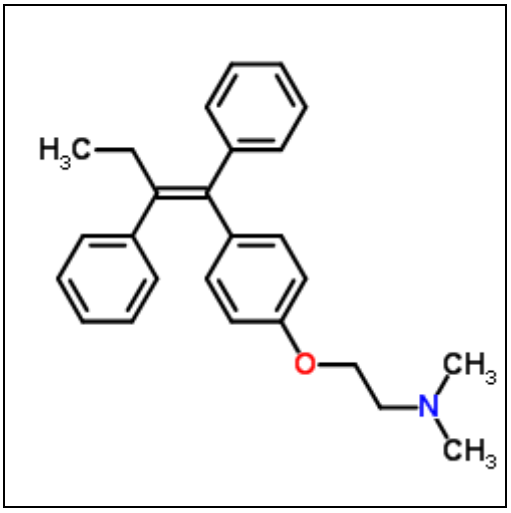
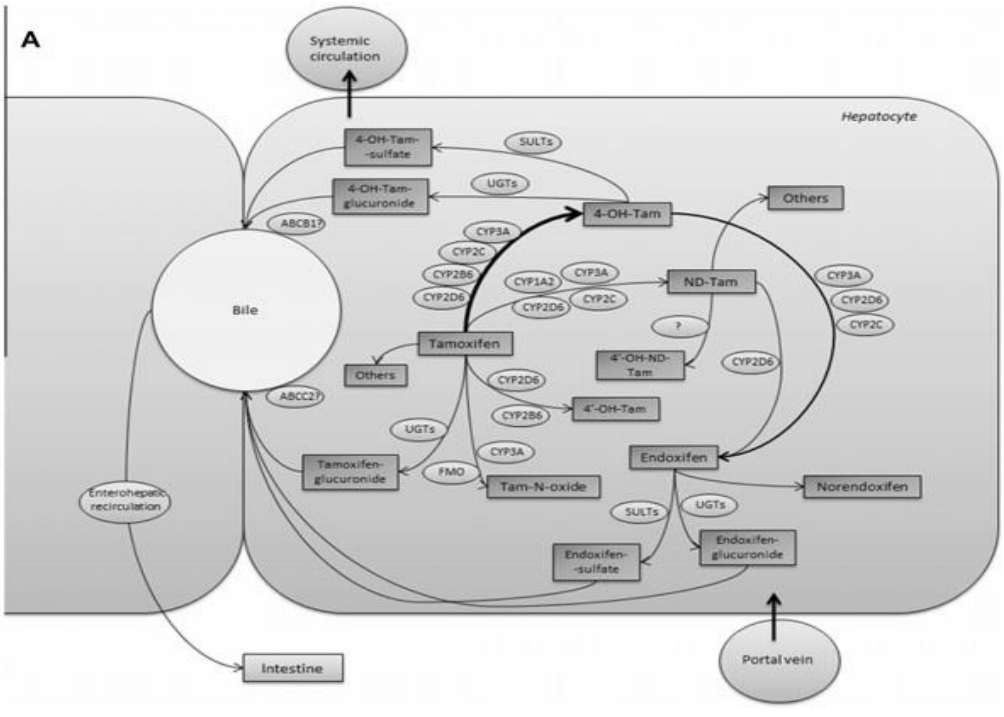


Figure (5): The chemical structures of tamoxifen [25].

Tamoxifen Metabolism:

Tamoxifen is a prodrug, requiring cytochrome P450 enzyme-mediated metabolism to form the active metabolite endoxifen [26]. Tamoxifen is transformed predominantly by the drug- metabolizing enzymes CYP3a4 and CYP2D6 into the therapeutically more efficient drug metabolites 4-hydrox y tamoxifen (4-OH-tamoxifen) and endoxifen . By binding to the er α (erα) tamoxifen and its metabolites modulate, the estrogen-induced transcription of erα target genes. the metabolites 4-OH-tamoxifen and endoxifen show up to 100 times higher affinity to the erα than the parental compound . as a result, the efficacy of tamoxifen strongly depends on its appropriate bioactivation by cytochrome P450 enzymes. CYP2D6 is highly polymorphic and shows a high interindividual variability in its activity . Other enzymes involved in tamoxifen metabolism comprise CYP2C9, CYP2C19 and CYP2B6 . these three enzymes are also involved in the formation of 4-OH-tamoxifen and endoxifen, but their contribution may depend on actual tamoxifen concentrations and on CYP2D6 activity [27, 28].



ISSN: 0975-8585

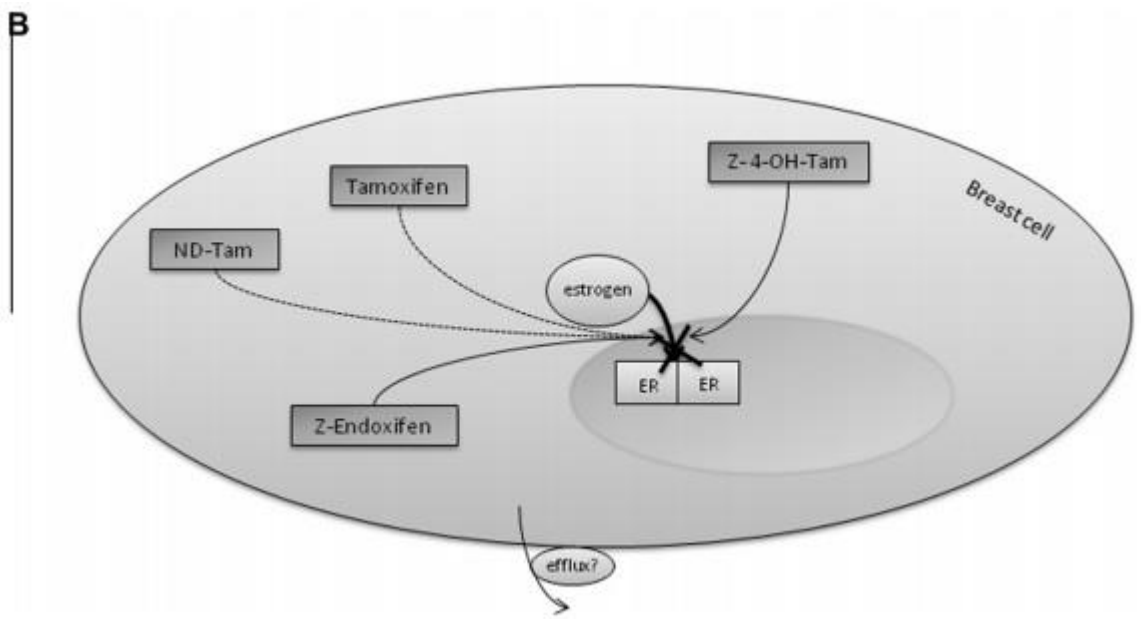


Figure (6):The hepatic metabolism of tamoxifen[29].

CONCLUSION

Tamoxifen (TAM) is an important anticancer drug that is commonly used in the prevention and treatment of breast cancer, and also exhibits antioxidant and cardioprotective effects. One mechanism by which TAM inhibits cancer cell growth is competitive blocking of estrogen receptors. Tamoxifen is a prodrug , requiring cytochrome P450 enzyme-mediated metabolism to form the active metabolite endoxifen. tamoxifen is transformed predominantly by the drug- metabolizing enzymes CYP3a4 and CYP2D6 into the therapeutically more efficient drug metabolites 4-hydrox y tamoxifen (4-OH-tamoxifen) and endoxifen

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