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New insights into the metabolism of tamoxifen and its role in the treatment and prevention of breast cancer

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Abstract

The metabolism of tamoxifen is being redefined in the light of several important pharmacological observations. Recent studies have identified 4-hydroxy N-desmethyl tamoxifen (endoxifen) as an important metabolite of tamoxifen necessary for antitumor actions. The metabolite is formed through the enzymatic product of *CYP2D6* which also interacts with specific selective serotonin reuptake inhibitors (SSRIs) used to prevent the hot flashes observed in up to 45% of patients taking tamoxifen. Additionally, the finding that enzyme variants of *CYP2D6* do not promote the metabolism of tamoxifen to endoxifen means that significant numbers of women might not receive optimal benefit from tamoxifen treatment. Clearly these are particularly important issues not only for breast cancer treatment but also for selecting premenopausal women, at high risk for breast cancer, as candidates for chemoprevention using tamoxifen.

Keywords

selective serotonin reuptake inhibitors; raloxifene; selective estrogen receptor modulators; ospemifene; arzoxifene

Introduction

The aim of the body's biotransformation mechanisms is to prevent potentially toxic xenobiotic substances that include drugs, from damaging the body. That being the case, an orally active medicine must overcome numerous challenges to reach a target organ and produce the appropriate pharmacological effect at a receptor system. There is not one but several stages of biotransformation of a lipophilic drug such as tamoxifen that are designed to enhance the hydrophilic nature of the chemical so it can be rapidly eliminated. The stages of biotransformation are called phases I, II and III.

Phase I metabolism enhances the water solubility of a lipophilic chemical by hydroxylating an aromatic compound to become a phenol or hydrolyzing an esterified compound. These reactions are conducted by the family of cytochrome P_{450} enzymes referred to as CYP's. Phase II metabolism further increases the water solubility of the Phase I product by attaching highly water soluble entities. In the case of selective estrogen receptor modulators (SERMs) sugars (glucuronic acid) and salts (sulfates) are the most important conjugation products. In contrast,

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the phase III system are efflux pump molecules (also known as p-glycoproteins and multi-drug resistance transports protein) that exclude unmetabolized drugs from the epithelial cells of the intestinal tract immediately upon absorption.

In general terms, the ingested SERM must survive "first pass" metabolism from the intestine to the liver to have any chance of reaching target organs around the body. The general principles are illustrated in Figure 1 where the SERM is biotransformed by CYPs in the intestinal wall and Phase II metabolism occurs via intestinal bacteria. A fraction of the administered dose is then absorbed into the hepatic portal vein and further biotransformed by phase I CYPs and/or glucuronidated or sulfated in phase II metabolism in the liver. By way of example, only 2% of the administered raloxifene survives and is bioavailable for systemic distribution[1].

Tamoxifen, the first SERM

The nonsteroidal antiestrogen tamoxifen (ICI 46,474 Nolvadex$_{®}$) is a pioneering medicine [2] used to treat all stages of breast cancer in more than 120 countries throughout the world. The compound ICI 46,474 was discovered in the Fertility Control Program at Imperial Chemical Industries (ICI Pharmaceuticals Division, now AstraZeneca) in Alderley Park, Cheshire, England in the early 1960's [3–5]. The drug was found to be an extremely potent postcoital contraceptive in the rat [4,5]. Unfortunately, ICI 46,474 did not exhibit antifertility properties in women, in fact, quite the opposite, it induced ovulation [6,7]. As a result, the medicine was, at one time, marketed in the United Kingdom for the induction of ovulation in subfertile women with a functional hypothalamo-pituitary-ovarian axis.

There is a known link between estrogen and the initiation and growth of some breast cancers [8] so the nonsteroidal antiestrogen ICI 46,474 was tested as a potential treatment for advanced breast cancer in postmenopausal women. The antiestrogen produced response rates of 25–35% in unselected patients comparable to diethylstilbestrol and high dose androgen therapy, the standard endocrine therapies at the time [9,10]. However, fewer side effects were noted with tamoxifen [9,10]. As a result, the drug was approved as a palliative option for the hormonal treatment of breast cancer in the UK in 1973. There the story may have ended had not tamoxifen been reinvented as the first targeted therapy for breast cancer[2].

The seminal observations by Elwood Jensen that estrogen action is mediated by the estrogen receptor (ER)[11,12] in its target tissues (uterus, vagina pituitary and breast tumors) opened the door to targeting tamoxifen to select patients with the ER in their metastatic tumor[13, 14]. However, a strategic plan was developing to use tamoxifen in a broader range of patient populations. Laboratory studies conducted in the 1970's showed that tamoxifen blocked estrogen binding to the ER [15–17], should be used as a long-term adjuvant therapy to suppress tumor recurrence [18–20] and the drug also had potential as a chemopreventive agent [21, 22].

Clinical studies subsequently confirmed that long-term adjuvant tamoxifen therapy, targeted to the patients with ER positive breast cancers, significantly decreased the death rate from the disease [23] and contributes to the current decline in death from breast cancer nationally [24]. Overall, the strategy of targeted long-term "antiestrogenic" [25] treatment for breast cancer has presaged the current fashion of targeting anticancer agents to other organ sites in the body.

Despite the fact that aromatase inhibitors show superiority over tamoxifen as adjuvant therapy in postmenopausal women[26–29], several issues have surfaced that have retained tamoxifen as a useful therapeutic agent worldwide. The medicine is extremely cheap compared to aromatase inhibitors so tamoxifen remains an essential anticancer agent in undeveloped countries or in countries with under-funded managed healthcare systems. Furthermore, tamoxifen is the only appropriate antiestrogenic therapy for premenopausal women whether

they are being treated for breast cancer or whether chemoprevention is being considered[30]. For these reasons, new knowledge that can enhance the appropriate use of an established drug is of value to improve healthcare.

There are current initiatives to translate emerging knowledge on genetic variations in drug metabolism to target patient populations.[31] It is reasoned that by applying pharmacogenomic tests to specific patient populations, there will be fewer surprises with side effects, drug interactions, and a higher probability of increasing therapeutic effectiveness in the treatment or prevention of disease. The promise of practical progress is exemplified in this article using tamoxifen as the model drug.

Tamoxifen is a prodrug and can be metabolically activated to 4-hydroxytamoxifen[32–34] or alternatively can be metabolically routed via N-desmethyltamoxifen to 4-hydroxy-N-desmethyltamoxifen [35,36] (Figure 2). The hydroxy metabolites of tamoxifen have a high binding affinity for the ER[32,37]. The finding that the enzyme produced by *CYP2D6* activates tamoxifen to hydroxylated metabolites 4-hydroxytamoxifen and endoxifen[38] has implications for cancer therapeutics. Women with enzyme variants that cannot make endoxifen may not have as successful an outcome with tamoxifen therapy. Alternatively, women who have a normal enzyme may make high levels of the potent antiestrogen endoxifen and experience hot flashes. As a result, these women may take selective serotonin reuptake inhibitors (SSRIs) to ameliorate hot flashes but there are potential pharmacological consequences to this strategy. Some of the SSRIs are metabolitically altered by the *CYP2D6* enzyme product[39]. It is therefore possible to envision a drug interaction whereby SSRIs block the metabolic activation of tamoxifen.

This article will describe the scientific twists and turns that tamoxifen and its metabolites have taken over the past 30 years. The story is naturally dependent on the fashions in therapeutic research at the time. What seems obvious to us as a successful research strategy today, with millions of women taking tamoxifen, was not so 30 years ago at the beginning when the clinical community and pharmaceutical industry did not see “antihormones” as a priority at all for drug development[25]. In 1972, tamoxifen was declared an orphan drug with no prospects[2].

Basic mechanisms of tamoxifen metabolism

The original survey of the putative metabolites of tamoxifen was conducted in the laboratories of ICI Pharmaceuticals Division and published in 1973 [40]. A number of hydroxylated metabolites were noted (Figure 3) following the administration of ^{14}C labeled tamoxifen to various species (rat, mouse, monkey, and dog). The major route of excretion of radioactivity was in the feces. The rat and dog were used to show that up to 53% of the radioactivity derived from tamoxifen was excreted via the bile and up to 69% of this was reabsorbed via a enterohepatic recirculation until eventual elimination occurs[40]. The hydroxylated metabolites are excreted as glucuronides. However, no information about their biological activity was available until the finding that 4-hydroxytamoxifen had a binding affinity for the ER equivalent to 17β estradiol [32]. Similarly, 3,4 dihydroxytamoxifen (Figure 3) bound to the human ER but interestingly enough, 3,4 dihydroxytamoxifen was not significantly estrogen-like in the rodent uterus despite being antiestrogenic [32].

Additional studies on the metabolism of tamoxifen in four women [41] identified 4-hydroxytamoxifen as the primary metabolite using a thin layer chromatographic technique to identify ^{14}C labeled metabolites. This assumption, coupled with the potent antiestrogenic actions of 4-hydroxytamoxifen [32] and the conclusion that it was an advantage, but not a requirement for tamoxifen to be metabolically activated [33,42] seemed to confirm the idea that 4-hydroxytamoxifen was the active metabolite that bound in rat estrogen target tissues to block estrogen action[34]. However, the original analytical methods used to identify 4-

hydroxytamoxifen as the major metabolite in humans were flawed[43] and subsequent studies identified N-desmethyltamoxifen (Figure 4) as the major metabolite circulating in human serum[44]. The metabolite was found to be further demethylated to N-desdimethyltamoxifen (Metabolite Z)[45] and then deaminated to Metabolite Y, a glycol derivative of tamoxifen [46,47]. The metabolites (Figure 4) that are **not** hydroxylated at the 4 position of tamoxifen (equivalent to the 3 phenolic hydroxyl of estradiol) are all weak antiestrogens that would each contribute to the overall antitumor actions of tamoxifen at the ER based on their relative binding affinities for the ER and their actual concentrations locally.

At the end of the 1980's the identification of another metabolite tamoxifen 4-hydroxy N-desmethyltamoxifen in animals[48] and man [35,36] was anticipated but viewed as obvious and uninteresting. The one exception that was of interest was Metabolite E (Figure 3) identified in the dog [40]. This phenolic metabolite without the dimethylaminoethyl side chain is a full estrogen[47,49]. The dimethylaminoethoxy side chain of tamoxifen is necessary for antiestrogenic action[49].

It is not a simple task to study the actions of metabolites *in vivo*. Problems of pharmacokinetics, absorption and subsequent metabolism all conspire to confuse the interpretation of data. Studies *in vitro* using cell systems of estrogen target tissues were defined and refined in the early 1980's to create an understanding of the actual structure function relationships of tamoxifen metabolites. Systems were developed to study the regulation of the prolactin gene in primary cultures of immature rat pituitary gland cells[42,50] or cell replication in ER positive breast cancer cells[51–54]. Overall, these models were used to describe the importance of a phenolic hydroxyl to tether a triphenylethylenes appropriately in the ligand binding domain of the ER and to establish the appropriate positioning of an "antiestrogenic" side chain in the "antiestrogen region" of the ER[50] to modulate gene activation and growth[42,50,55–58]. These structure function studies, that created hypothetical models of the ligand-ER/complex, were rapidly advanced with the first reports of the x-ray crystallography of the estrogen, 4-hydroxytamoxifen[59] or raloxifene ER[60] complexes. The ligand-receptor protein interaction was subsequently interrogated by examining the interaction of the specific amino acid, asp 351 with the antiestrogenic side chain of the ligand[61]. A mutation was found as the dominant ER species in a tamoxifen-stimulated breast tumor grown in athymic mice[61,62]. The structure function relationships studies, that modulated estrogen action at a transforming growth factor alpha gene target, demonstrated that the ligand shape would ultimately program the shape of the ER complex in a target tissue [30,63–65]. This concept is at the heart of metabolite pharmacology and is required to switch on and switch off target sites around the body. The other piece of the mechanism of SERMs puzzle that was eventually solved was the need for another player to partner with the ER complex. Coactivators[66] can enhance the estrogen-like effects of compounds at a target site[67]. However, in the early 1990's, the molecular and clinical use of this knowledge with the development and application of SERMs was in the future[68].

The urgent focus of translational research in the early 1990's was to discover why tamoxifen was a complete carcinogen in rat liver[69,70] and to determine whether there was a link between metabolism and the development of endometrial cancer noted in very small but significant numbers of postmenopausal women taking adjuvant tamoxifen[71,72].

All interest in the metabolism of tamoxifen focused on the production of DNA adducts[73] that were responsible for rat liver carcinogenesis and, at the time, believed to be potentially responsible for carcinogenesis in humans[74]. Although many candidates were described [75–78], the metabolite found to be responsible for the initiation of rat liver carcinogenesis is α-hydroxytamoxifen[79–83] (Figure 5) Alpha-hydroxytamoxifen has been resolved into R-(+) and S- (−) enantiomers. Metabolism by rat liver microsomes gave equal amounts of the

two forms, but in hepatocytes the R form gave 8x the level of DNA adducts as the S form. As both had the same chemical reactivity towards DNA, Osborne and coworkers[84] suggested that the R form was a better sulfotransferase substrate. This enzyme is believed to catalyze DNA adduct formation. Subsequently, Osborne and coworkers[85] conducted studies with alpha-hydroxy-N-desmethyltamoxifen; the R-(+) gave 10x the level of adducts in rat hepatocytes as the S-(−).

There were reasonable concerns that the hepatocarcinogenicity of tamoxifen in rats would eventually translate to humans but fortunately this is now known to be untrue[86]. The demonstration of carcinogenesis in the rat liver appears to be related to poor DNA repair mechanisms in the inbred strains of rats. In contrast, it appears that the absence of liver carcinogenesis in women exposed to tamoxifen [87] is believed to result from the sophisticated mechanisms of DNA repair inherent in humans cells..

It is clear from this background about the early development of tamoxifen and the fact that tamoxifen was considered to be such a safe drug in comparison to other cytotoxic agents used in therapy during the 1970's and 1980's, that there was little enthusiasm for in-depth studies of tamoxifen metabolism. However, this perspective was to change in the 1990's with the widespread use of tamoxifen as the gold standard for the treatment and prevention of breast cancer. Questions needed to be addressed: 1) what happens to tamoxifen in patients? and 2) can improvements be made to the molecule?

Clinical pharmacology

A number of analytical techniques are available to evaluate blood levels of tamoxifen and its metabolites once the drug is absorbed. The early method of thin layer chromatography, and the current method of high performance liquid chromatography (HPLC) both depend on the conversion of the triphenylethylenes to fluorescent phenanthrenes for their detection (Figure 6). The original description of the reaction [88] was successfully adapted [89] to identify tamoxifen, N-desmethytamoxifen and 4-hydroxytamoxifen in plasma samples.

Subsequent improvements were made [90] but the method significantly underestimated phenolic metabolites (4-hydroxytamoxifen) and had no internal standardization. In contrast, a method of post column fluorescence activation [91] or preliminary purification from interfering substance using a Sep-Pack C18 cartridge (Waters Association, Milford MA) [92] with internal standardization considerably improved accuracy. The detection of tamoxifen metabolites in serum was further improved by Lien and coworkers [93] and recently by Lee and coworkers [94] who adapted the methods [95,96] developed to perform "on line" extraction and post column cyclization. Using this methodology the limits of detection for 4-hydroxy tamoxifen and endoxifen are 0.5 and 0.25 ng/ml respectively [97]. Since there was such initial controversy about the identification of metabolites in patient serum, it is perhaps important to describe the validation of 4-hydroxy-desmethyltamoxifen as a metabolite of tamoxifen in patients. Tamoxifen metabolites were investigated in a 57 year old female patient receiving tamoxifen treatment[35]. Two major chromatographic peaks were identified in bile following treatment with β-glucuronidase. On major peak co-elevated with 4-hydroxytamoxifen but the second peak was proven to be 4-hydroxy-N-desmethyltamoxifen using a) co-elution with an authentic standard on reversed-phase chromatography and formation of fluorescent derivative by cyclization; b), the detection of a molecular ion (M+l)+ of 374 m/2 as determined by liquid chromatography-mass spectrometry; and c) a fragmatogram identical to that of the authentic standard, obtained by mass spectrometry. Subsequent refinement of the technology improved detection for identification of 4-hydroxy-N-desmethyltamoxifen in human serum, tissues[36] and rat tissues[93].

Studies confirm that tamoxifen is 98% bound to serum albumin which ultimate creates a long biological half life (plasma half life 7 days)[93]. A single oral dose of 10 mg tamoxifen (half the daily dose) produces peak serum levels of 20–30 ng of tamoxifen/ml within 3–6 hours but it must be stressed that patient variation is very large [98]. Nevertheless, continuous therapy with either 10mg bid [98] or 20 mg bid [99] produces steady state levels within 4 weeks. Blood levels of tamoxifen can average around 150 ng/ml for 10 mg tamoxifen bid and 300 ng/ml for 20 mg tamoxifen bid. A strategy of using loading doses [98,100] to elevate blood levels rapidly has not produced any therapeutic benefit.

Overall, the results from the metabolic studies with tamoxifen during the 1970's and 80's did not help clinicians to use tamoxifen more effectively. The structures of metabolites were in fact used as leads to create new molecules for clinical development.

Metabolic Mimicry

The demonstration [32] that the class of compounds referred to as nonsteroidal antiestrogens were metabolically activated to compounds with high binding affinity for the ER created additional opportunities for the medicinal chemists within the pharmaceutical industry to develop new agents. This was particularly true once the nonsteroidal antiestrogens were recognized to be SERMs [101–103] and had applications not only for the treatment and prevention of breast cancer but also as potential agents to treat osteoporosis and coronary heart disease[104,105]. The reader is referred to other recent review articles to obtain further details of new medicines under investigation [104,105] but some current examples are worthy of note and will be mentioned briefly. Compounds of interest that have their structural origins as metabolites from nonsteroidal antiestrogens are summarized in Figure 7. Raloxifene is an agent that originally was destined to be a drug to treat breast cancer but it failed in that application [106]. It appears that the pharmacokinetics and bioavailability of raloxifene are a challenge. Only about 2% of administered raloxifene is bioavailable [1] but despite this, the drug is known to have a long biological half life of 27 hr. The reason for this disparity is that raloxifene is a polyphenolic drug that can be glucuronidated and sulfated by bacteria in the gut so the drug cannot be absorbed[107,108]. This phase II metabolism in turn controls enterohepatic recirculation and ultimately impairs the drug from reaching and interacting with receptors in the target. This concern has been addressed with the development of the long-acting raloxifene derivative arzoxifene that is known to be superior to raloxifene as a chemopreventive in rat mammary carcinogenesis. [109]. One of the phenolic groups (Figure 7) is methylated to provide protection from Phase II metabolism. Nevertheless, arzoxifene has not performed well as a treatment for breast cancer [110,111]; higher doses are less effective than lower doses. These data imply that effective absorption is impaired by phase III metabolism. That being said, the results of trials evaluating the effects of arzoxifene as a drug to treat osteoporosis, using lower doses, are eagerly awaited. Perhaps arzoxifene will be a better breast cancer preventive than a treatment.

Unfortunately, the bioavailability of phenolic drugs is also dependent on Phase II metabolism to inactive conjugates in the target tissue. 4-Hydroxytamoxifen,[32] is only sulfated by three of seven sulfotransferase isoforms whereas raloxifene is sulfated by all seven [112]. Maybe local phase II metabolism plays a role in neutralizing the antiestrogen action of raloxifene in the breast. Falany and coworkers [112] further report that SULT1E1, that sulfates raloxifene in the endometrium, is only expressed in the secretory phase. In contrast, 4-hydroxytamoxifen is sulfated at all stages of the uterine cycle.

Lasofoxifene is a diaryltetrahydronaphthalene derivative referred to as CP336156 [113] that has been reported to have high binding affinity for ER and have potent activity in preserving bone density in the rat[114,115]. The structure of CP336156 is reminiscent of the putative

antiestrogenic metabolite of nafoxidine[116] that failed to become a breast cancer drug because of unacceptable side effects[117]. There are two disasterometiric salts of the chemical shown in Figure 7. CP336156 is the *l* enantiomer that has 20 times the binding affinity for the ER as the *d* enantiomer. Studies demonstrate that the *l* enantiomer had twice the bioavailbility of the *d* enantiomer. The authors [113] ascribed the difference to enantioselective glucuronidation of the *d* isomer. An evaluation of CP336156 in the prevention and treatment of rat mammary tumors induced by N-nitroso-N-methylurea shows activity similar to that of tamoxifen[118].

Ospemifene or deaminohydroxytoremifene is related to metabolite Y formed by the deamination of tamoxifen[47]. Metabolite Y has a very low binding affinity for the ER[47, 119] and has weak antiestrogenic properties compared with tamoxifen. Ospermifene is a known metabolite of toremifene (4 chlorotoremifene) but unlike tamoxifen, there is little carcinogenic potential in animals[120]. It is possible that the large chlorine atom on the 4 position of toremifene and ospermifene reduces α hydroxylation to the ultimate carcinogen related to α hydroxy tamoxifen (Figure 6) D eaminohydroxytoremifene has very weak estrogenic and antiestrogenic properties in vivo[121] but demonstrates SERM activity in bone and lowers cholesterol. The compound is proposed to be used as a preventative for osteoporosis. Preliminary clinical data in healthy men and postmenopausal women demonstrate pharmacokinetics suitable for daily dosing between 25 and 200 mg[122]. Interestingly enough, unlike raloxifene, ospermifene has a strong estrogen-like action in the vagina but neither ospermifene nor raloxifene affect endometrial histology[123,124]. Overall, the goal of developing a bone specific agent is reasonable, but the key to commercial success will be the prospective demonstration of the prevention of breast and endometrial cancer as beneficial side effects. This remains a possibility based on prevention studies completed in the laboratory [125,126].

Tamoxifen Metabolism Today

A comprehensive evaluation of the sequential biotransformation of tamoxifen has been completed by Desta and colleagues[38]. They used human liver microsomes and experiments with specifically expressed human cytochrome P450's to identify the prominent enzymes involved in Phase I metabolism. Their results are summarized in Figure 2 with the relevant *CYP* genes indicated for the metabolic transformations. The authors make a strong case that N-desmethyltamoxifen, the principal metabolite of tamoxifen that accumulates in the body, is converted to endoxifen by the enzymatic product of *CYP2D6*. The *CYP2D6* product is also important to produce the potent primary metabolite 4-hydroxytamoxifen but the metabolite can also be formed by the enzymatic products: *CYP2B6, CYP2C9, CY2C19* and *CYP3A4*.

The *CYP2D6* phenotype is defined as the metabolic ratio (MR) by dividing the concentration of an unchanged probe drug, known to be metabolized by the CYP2D6 gene product, by the concentration of the relevant metabolite at a specific time. These measurements have resulted in the division of the *CYP2D6* phenotype in four metabolic classes; poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizes (UM). Over 80 different single nucleotide polymorphisms have been identified but there are inconsistencies in the precise definitions of the ascribing a genotype to a phenotype[127, 128]. Bradford[128] and Raimondo and coworkers[129] have described the frequency of common alleles for *CYP2D6.* Pertinent to the current discussion of tamoxifen metabolism, the *CYP2D6*4* allele[130] is estimated to have a frequency of 12–23% in Causasians, 1.2–7% in black Africans and 0–2.8% in Asians[127,128]. A lower estimate of (<10%) of the PM phenotype is presented by Bernard and coworkers[131].

The molecular pharmacology of endoxifen has recently been reported [37,132,133]. Endoxifen and 4-hydroxytamoxifen were equally potent at inhibiting estrogen stimulated growth of ER

positive breast cancer cells MCF-7, T47D and BT474. Both metabolites are significantly superior *in vitro* to tamoxifen the parent drug. Additionally, the estrogen-responsive genes pS_2 and progesterone receptor were both blocked to an equivalent degree by endoxifen and 4-hydroxytamoxifen[132,133]. Lim and co-workers[133] have extended the comparison of endoxifen and 4-hydroxytamoxifen in MCF-7 cells by comparing and contrasting global gene regulation using the Affymetrix U133A Gene Chip Array. There were 4062 total genes that were either up or down regulated by estradiol whereas, in the presence of estradiol, 4-hydroxytamoxifen or endoxifen affected 2444 and 2390 genes respectively. Overall, the authors[133] demonstrated good correlation between RTPCR and select genes from the microarray and concluded that the global effects of endoxifen and 4-hydroxytamoxifen were similar.

Stearns and coworkers[97] and Jin and coworkers[134] have confirmed and significantly extended Lien's original identificatiaon of endoxifen and observation[35,36] that there are usually higher circulating levels of endoxifen than 4-hydroxytamoxifen in patients receiving adjuvant tamoxifen therapy. However, Flockhart's group[97] have advanced the pharmacogenomics and drug interactins surrounding tamoxifen therapy that should be a consideration in the antihormonal treatment of breast cancer.

The ubiquitous use of tamoxifen for the treatment of node negative women[135] during the 1990's, the use of tamoxifen plus radiotherapy following lumpectomy for the treatment of ductal carcinoma in situ (DCIS)[136] as well as the option to use tamoxifen for chemoprevention in high risk pre and postmenopausal women[137] enhanced awareness of the menopausal side effects experienced by women when taking tamoxifen. Up to 45% of women with hot flashes grade them as severe[137] therefore there have been efforts to improve quality of life. Treatments with the SSRIs are popular [97,138,139] (Figure 8). The SSRIs are twice as effective as the "placebo" effect at reducing menopausal symptoms in randomized clinical trials[138–140], so there is naturally an increased usage of SSRIs with long-term tamoxifen treatment to maintain compliance. Unfortunately, the metabolism of tamoxifen to hydroxylated metabolites[141–143] and the metabolism of SSRIs[39,144–147] both occur via the *CYP2D6* gene product. Indeed Stearns and coworkers[97] showed that the SSRI inhibitor paroxetine reduced the levels of endoxifen during adjuvant tamoxifen therapy and endoxifen levels decrease by 64% in women with wild type *CYP2D6* enzyme. Patients were examined who were taking venlafaxine, sertraline, and paroxetine and compared with those women who were homozygotes for the *CYP2D6 *4/*4* inactive genotype. Patients with the wild type gene who took the most potent inhibitor paroxetine had serum levels of endoxifen equivalent to the patients with the aberrant *CYP2D6* gene. In fact, the clinical data were consistent with the inhibition constants for the inhibition of *CYP2D6* by paroxetine (potent), fluoxetine, sertraline, citalopram (intermediate) and venlafaxine (weak) which are 0.05, 0.17, 1.5, 7 and 33μmol/L respectively.

The *CYP2D6* gene product that is fully functional (wild type) is classified as the *CYP2D6*1*. A large number of alleles are associated with no enzyme activity or reduced activity. Conversely, high metabolizers can have multiple copies of the *CYP2D6* allele[31]. A recent study by Borges[148] continues to expand our understanding of the detrimental effect of *CYP2D6* variants plus concomitant administration of SSRIs on endoxifen levels. But, it is the clinical correlations with tumor responses and side effects that are starting to provide clues about the importance of pharmacogenomics for tamoxifen to be optimally effective as a breast cancer drug.

Clinical Correlations

The significance of genotyping on clinical outcomes of a tamoxifen trial have been addressed using paraffin-embedded tumor blocks from a North Central Center Treatment Group (NCCTG) trial NCCTG 89-30-52[149]. The postmenopausal women with ER positive tumors received 5 years of adjuvant tamoxifen therapy. The tumor blocks were used to determine *CY2D6* (**4* and **6*) and *CYP3A5 (*3)* and *17* buccal swabs were used to test the veracity of the tumor genotyping. The concordance rate for the buccal swabs was 100%. Overall, the *CYP3A5*3* variant was not associated with any adverse clinical outcomes but the women with the *CYP2D6*4/*4* genotype had a higher risk of disease relapse but a lower incidence of side effects such as hot flashes.[149] The implication is that tamoxifen must be converted to endoxifen, a more potent antiestrogen.

In a follow up study[150] using the same database established for trial NCCTG 89-30-52, patient records were screened to determine the extent of SSRI prescribing. The goal was to establish the combined effect of genotyping and SSRI inhibition of the *CYP2D6* enzyme. Overall, the authors[150] concluded that a mutated *CYP2D6* gene or the inadvertent use of SSRIs that inhibit the *CYP2D6* enzyme product are independent predictors of breast cancer outcomes for postmenopausal women with breast cancer taking tamoxifen. In a recent complimentary study, Mortimer and coworkers[151] demonstrated that hot flashes were a strong predictor of positive outcomes for adjuvant tamoxifen treatment.

Although all of the current emphasis has been on the biological effects of tamoxifen in patients with the *CYPD6*4* variant, studies of *CYP3A5* 1* AND **3 1A1 *1* and *2* and *UGT2B15 ** and **2* have been undertaken and compared with carriers of *CYP2D6*4*. In contrast to the studies of Goetz and colleagues[149], patients who carry the *SULT1A1*1, CYP2D7*4* and *CYP3A5*3* alleles, and would be predicted to give rise to lower concentrations of metabolites with high affinity for the ER, might actually benefit from tamoxifen[152–155]. No differences were noted between genotypes *CYP2D6, SULT1A1* or *UGT 2B15* and tamoxifen treatment but Wegman and coworkers[155] claim that genetic variants of *CYP3A5* may predict response to tamoxifen. Clearly, reasons for the different conclusions need to be advanced. The hypothesis that variants of metabolizing enzymes can affect patient outcomes for the treatment of breast cancer must now be addressed in large populations and with prospective studies.

Conclusions

Overall, the study of tamoxifen metabolism has provided important clues which guided medicinal chemists to synthesize and develop new medicines. The study of metabolites has also provided valuable insight into the mechanism of action of SERMs at their target the ER. However, it is the recent research on the value of genotyping *CYPs* in breast cancer patients to improve response rates to tamoxifen therapy that is showing important promise. Genotyping patients for *CYP2D6* appears to be valuable to exclude the suboptimal use of tamoxifen in select individuals. Additionally, and perhaps more importantly, an effect of SSRIs on the blood levels of endoxifen has raised the possibility that the cheap and effective veteran tamoxifen could be targeted further to select populations of women to improve response rates. Avoiding SSRIs with a high affinity for *CYP2D6* gene product could improve tamoxifen's efficacy. Since tamoxifen is still the antihormonal treatment of choice for premenopausal patients and the only choice for breast cancer risk reduction in premenopausal women, then genotyping from buccal swabs appears to be a cheap and effective way of ensuring that tamoxifen is used to treat the appropriate woman.

It is necessary, however, to close on a note of caution. Very few patients have been studied to create definitive guidelines. That being said, the task of proving the value of these tantalizing

clues and hypotheses is the responsibility of clinicians to organize prospective clinical trials or at least there must be investment in the further analysis of archival material from randomized trials. The value of committing resources to establish hypothesis as fact is clear. An important cheap medicine should potentially be given only to women who will benefit from it. Indeed, it may be the role of *CYP2D6* in tamoxifen metabolism that is creating the small but significant advantage of aromatase inhibitors vs. tamoxifen in postmenopausal women.[26,27]. Again, this can be tested as the tumor blocks and patient records could be reviewed to determine genotyping and whether SSRIs were used. It would be remarkable to discover that the pharmacology of tamoxifen is undermining activity rather than the current view that aromatase inhibitors were better medicines because they have, unlike the SERMs, no estrogen-like actions at the level of the tumor.

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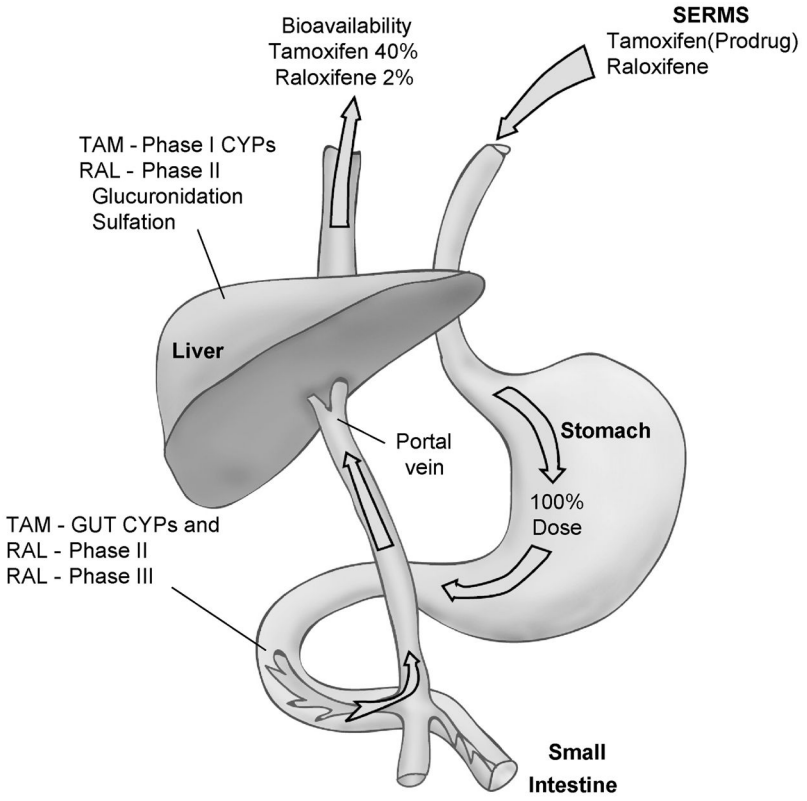

Figure 1.

The stylized representation of the absorption of two selective estrogen receptor modulators (SERMS) tamoxifen (TAM) or raloxifene (RAL) into the circulation as bioactive molecules. The polyphenolic SERM raloxifene must transverse phase II and phase III obstacles in the gut and the liver to get into the general circulation. This results in very little of the ingested drug being bioavailable at target sites. In contrast, tamoxifen is extremely lipophilic and 98% protein bound to serum albumin. This extends the duration of action of tamoxifen because phase II metabolism to phenolic compounds is retarded.

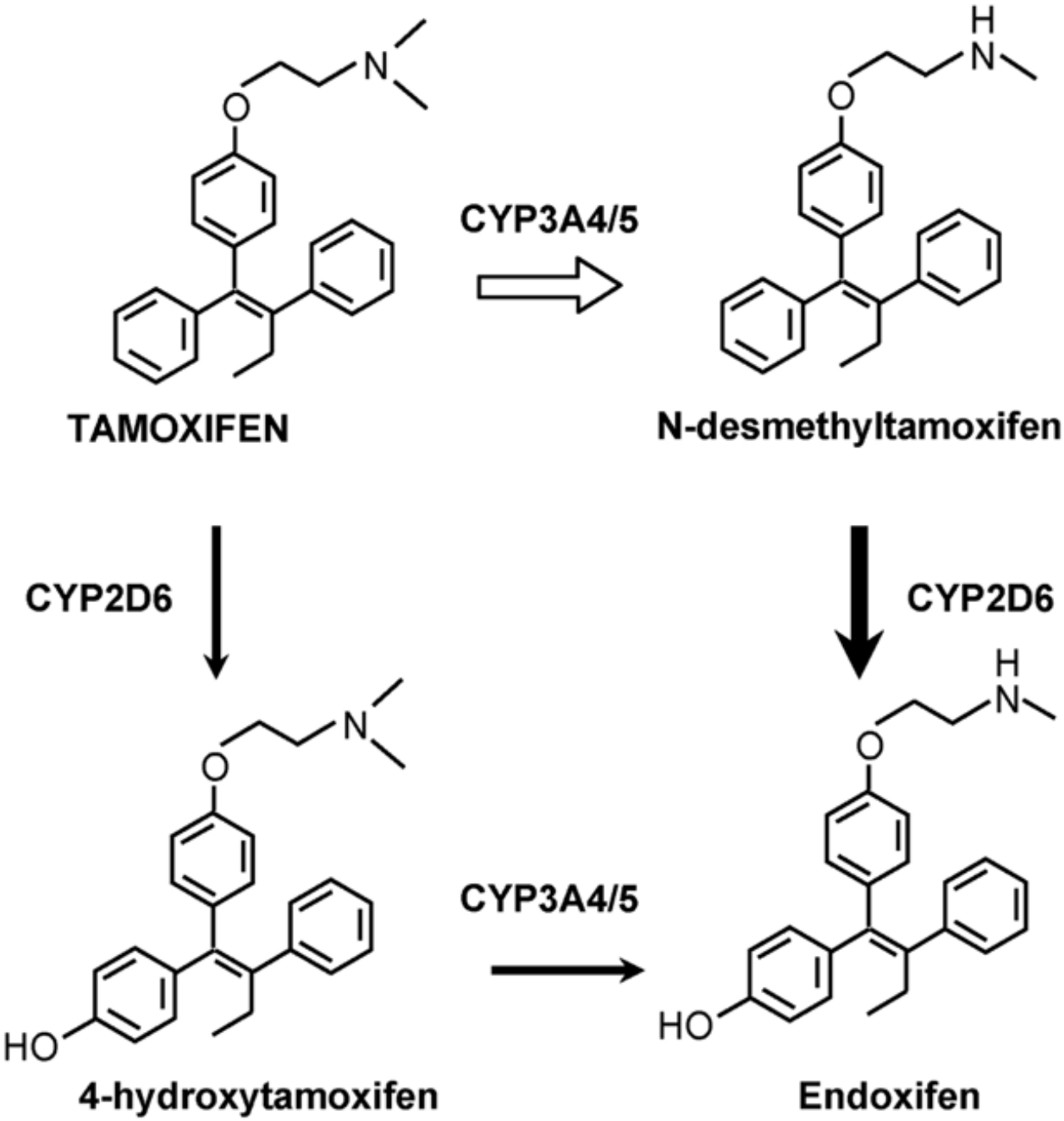

Figure 2.
The metabolic activation of tamoxifen to phenolic metabolites that have a high binding activity for the human estrogen receptor. Both 4-hydroxytamoxifen and endoxifen are potent antiestrogens *in vitro.*

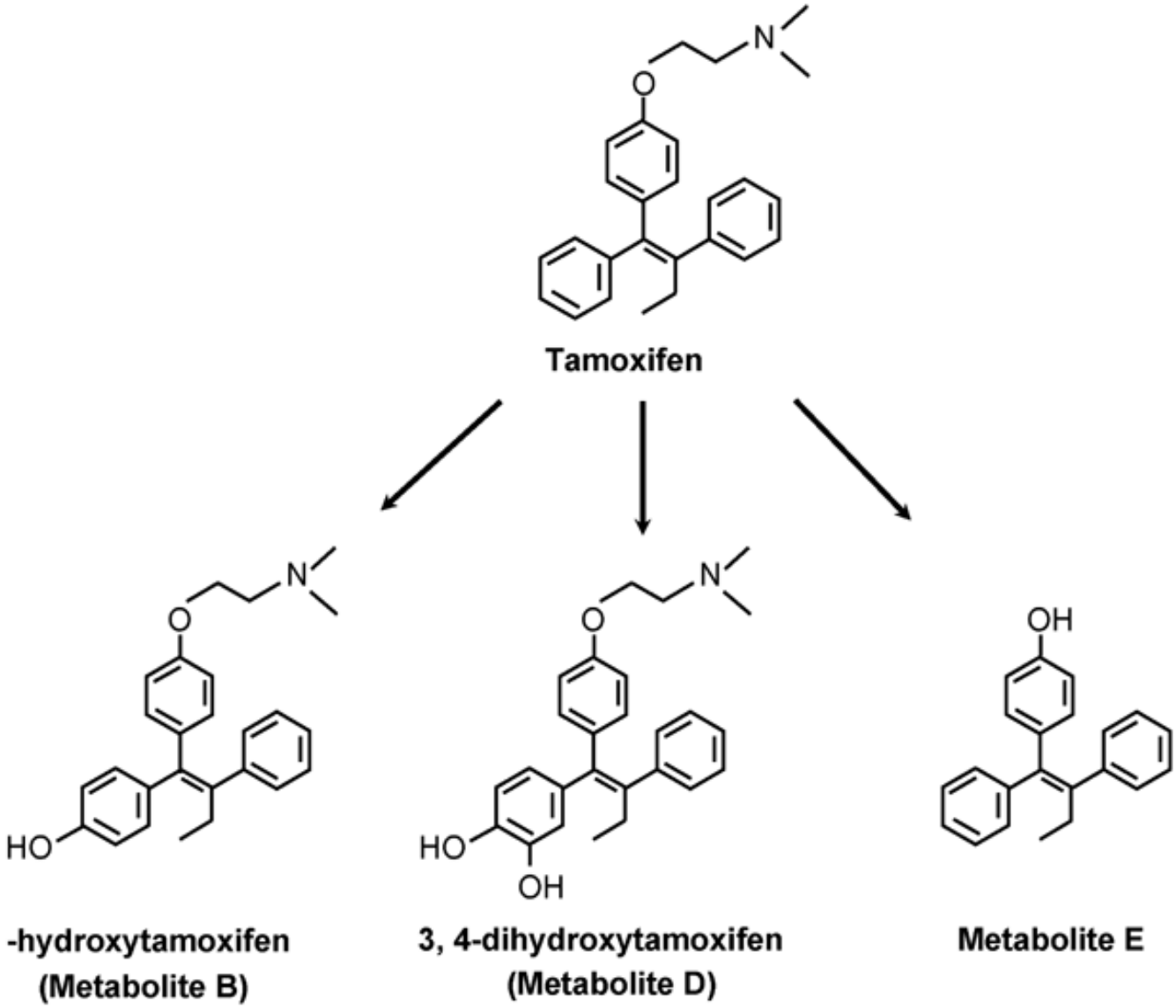

Figure 3.
The original hydroxylated metabolites of tamoxifen noted in animals by Fromson et al.[40]

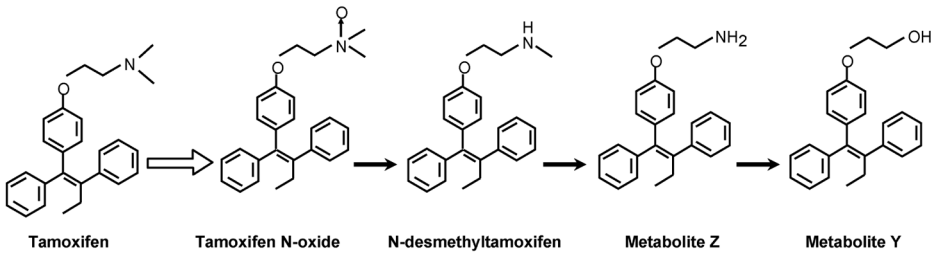

Figure 4.
The serial metabolic demethylation and deamination of the antiestrogenic side chain of tamoxifen. Each of the metabolites is a weak antiestrogen with poor binding affinity for the estrogen receptor.

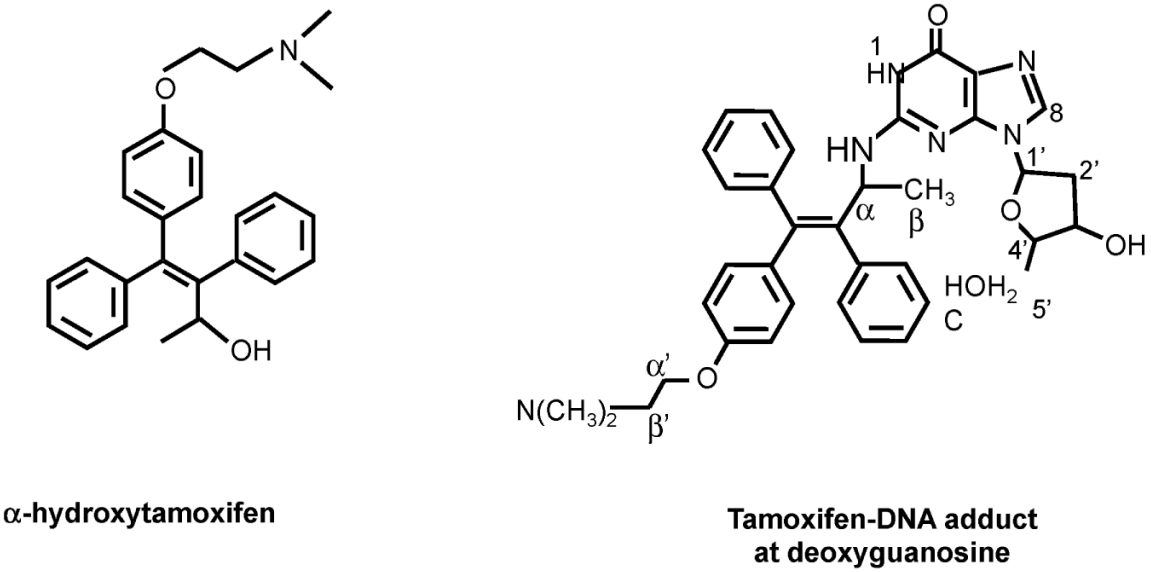

Figure 5.
The putative metabolite of tamoxifen, α hydroxytamoxifen that produces DNA adducts through covalent binding to deoxyguanosine.

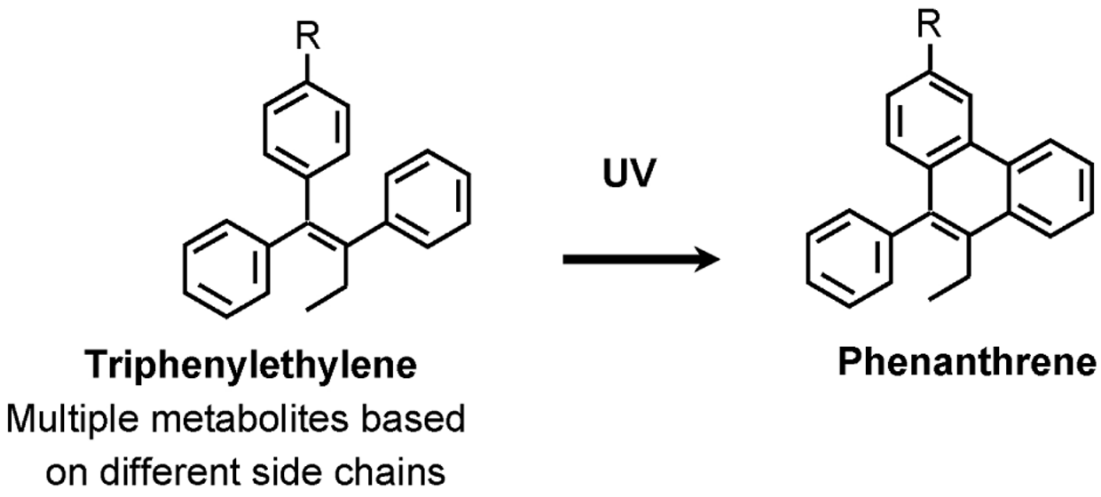

Figure 6.
The UV activation of a triphenylethylenes to a florescent phenanthrene. This basic reaction is exploited in the detection of serum tamoxifen levels.

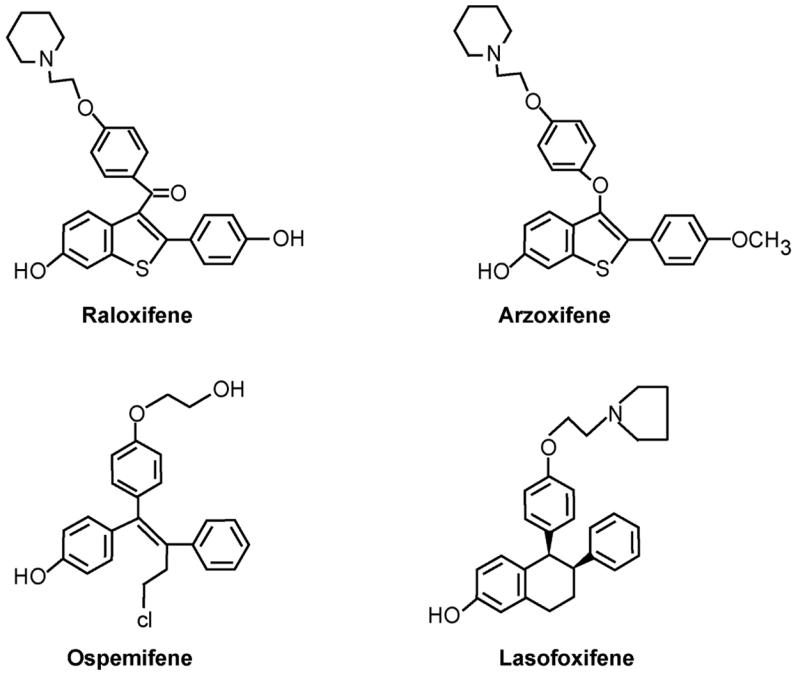

Figure 7.
The formulae of SERMs that have been developed based on the knowledge of the metabolic activation of tamoxifen (and nafoxidine, see text) as well as the metabolism of the antiestrogen side chain of tamoxifen to a glycol.

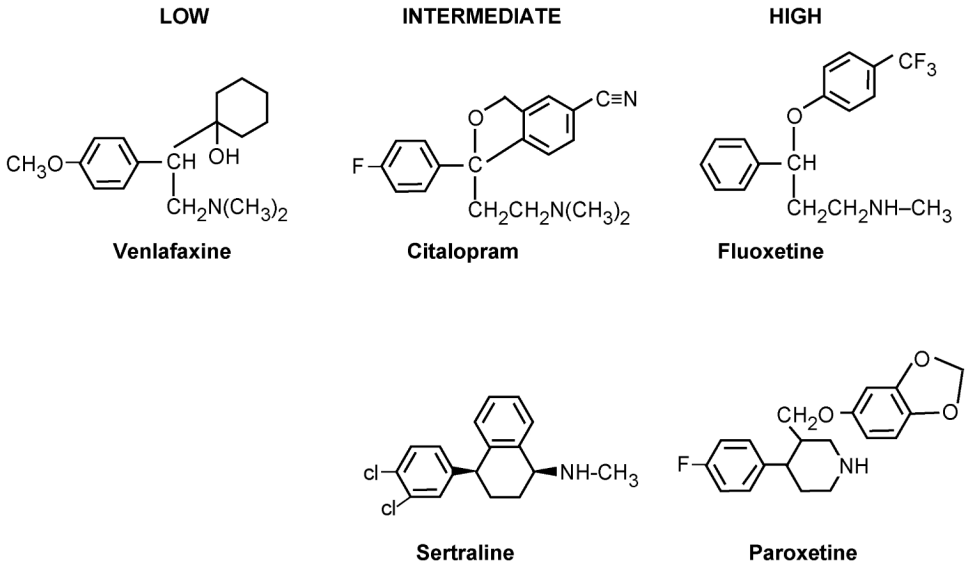

Figure 8.

The structures of selective serotonin reuptake inhibitors (SSRIs) that have low intermediate or high affinity for the *CYP2D6* enzyme system. High affinity binders for *CYP2D6* block the metabolic activation of tamoxifen to endoxifen (Figure 2).