Review article

Tamoxifen a pioneering drug: An update on the therapeutic potential of tamoxifen derivatives

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1. Introduction

Tamoxifen (ICI 46 474), trans-1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene, is the most commonly used drug for the treatment of estrogen receptor positive breast cancer and has been saving lives worldwide for the past four decades. Tamoxifen is considered a pioneering drug due to its ubiquitous use in both treatment and chemoprevention of breast cancer and also for research addressing novel selective estrogen receptor modulators (SERMs). Tamoxifen is cost effective, lifesaving, and devoid of major side effects in the majority of patients. The discovery of tamoxifen metabolites such as 4-hydroxy tamoxifen, N-desmethyl tamoxifen, and endoxifen has facilitated understanding of tamoxifen's and its metabolites' mechanisms of action in breast cancer therapy. Continuous efforts are being made by both industry and academia to synthesize novel tamoxifen derivatives in order to better understand the mechanism of this drug’s action and to generate new agents with reduced side effects for many therapeutic targets. This review article comprises the tamoxifen derivatives reported in the literature in the last few years and we anticipate that it will assist medicinal chemists in the synthesis of novel and pharmacologically potent agents for various therapeutic targets.

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ABSTRACT

Tamoxifen (ICI 46 474), trans-1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene, is the most commonly used drug for the treatment of estrogen receptor positive breast cancer and has been saving lives worldwide for the past four decades. Tamoxifen is considered a pioneering drug due to its ubiquitous use in both treatment and chemoprevention of breast cancer and also for research addressing novel selective estrogen receptor modulators (SERMs). Tamoxifen is cost effective, lifesaving, and devoid of major side effects in the majority of patients. The discovery of tamoxifen metabolites such as 4-hydroxy tamoxifen, N-desmethyl tamoxifen, and endoxifen has facilitated understanding of tamoxifen's and its metabolites' mechanisms of action in breast cancer therapy. Continuous efforts are being made by both industry and academia to synthesize novel tamoxifen derivatives in order to better understand the mechanism of this drug's action and to generate new agents with reduced side effects for many therapeutic targets. This review article comprises the tamoxifen derivatives reported in the literature in the last few years and we anticipate that it will assist medicinal chemists in the synthesis of novel and pharmacologically potent agents for various therapeutic targets.

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decades and progressed to become a significant part of our healthcare [11-14]. The story of the development of tamoxifen as a pioneering medicine for cancer treatment is very fascinating and inspiring for researchers worldwide who are working toward the development of novel medicines for various diseases. In the late 1950s, in the laboratories of Imperial Chemical Industries Ltd. Pharmaceutical division now known as AstraZeneca, a team with Dora Richardson (chemist), Michael J. K. Harper (reproductive endocrinologist), and Arthur L. Walpole (Head of Reproduction Research) was given the task of developing a post-coital contraceptive (the morning-after pill). Eventually, tamoxifen (Imperial Chemical Industries [ICI]) was invented and received marketing approval as a fertility treatment, but the drug never actually proved useful in human contraception. Walpole was very interested in exploring tamoxifen’s application in cancer research and treatment [5]. In 1972, Walpole collaborated with V.C. Jordan to conduct scientific research that led to the reinvention of the failed ICI 46 474 contraceptive to become tamoxifen, the first targeted agent for the treatment and prevention of breast cancer [6-11].

The “Father of Tamoxifen”, V. C Jordan, introduced the strategy of targeting estrogen receptor positive tumors with long term adjuvant tamoxifen therapy that increased the survival rate of hundreds of thousands of breast cancer patients around the world [12,13]. Tamoxifen is inexpensive and readily available to underfunded healthcare systems and that feature has caused an increase in its worldwide popularity as a miracle drug for breast cancer. Currently, tamoxifen is used for the treatment of all stages of estrogen receptor (ER) -positive (ER+) breast cancer in pre- and post-menopausal women in addition to hormone treatment for male breast cancer [14,15]. In addition, tamoxifen is used for the treatment of ductal carcinoma in situ and for the prevention of breast cancer in women at high risk of developing the disease [16,17]. Initially, tamoxifen was known as an anti-estrogen that reduced estrogen-induced effects by blocking estrogen receptors in breast tissues. Later, rigorous pharmacological investigation of tamoxifen provided evidence that tamoxifen acts as agonist at estrogen receptors in other body tissues such as endometrium, liver, and bone and thus, led to the development of a new drug group, the selective estrogen receptor modulators (SERMs) [18-23]. One of the important examples of a SERM is raloxifene (2) (Fig. 1), a failed breast cancer drug that has been successfully used to treat osteoporosis and prevent breast cancer in high risk post-menopausal women [24-27]. Although the benefits of tamoxifen are prominent, the use of tamoxifen is associated with increased risk of side effects such as hot flashes, menstrual abnormalities, uterine cancer and thromboembolic phenomena [28,29]. Since tamoxifen use is associated with an increase in cancer risks and unpleasant side effects, it is generally taken for five years followed by different therapeutics depending on the patient’s condition; furthermore, its use is not acceptable for all high-risk women. Further results from the Adjuvant Tamoxifen: Longer against Shorter (ATLAS) trial suggested that ten years of adjuvant tamoxifen therapy can reduce mortality to greater than five years [30].

Tamoxifen is marketed as a single Z (trans) isomer of p-β-dimethylaminoethoxy-1,2-diphenylbut-1-ene and is considered a lead compound that initiated the SERM development for the treatment of various diseases (such as osteoporosis, rheumatoid arthritis) and also for application of the SERM concept for all the members of the nuclear receptor family [31-38]. Pharmacological studies of tamoxifen in the human body suggested its conversion to three active metabolites: 1) 4-hydroxy tamoxifen (8); 2) N-desmethyltamoxifen (9); and 3) 4-hydroxy-N-desmethyltamoxifen, also known as endoxifen (10) (Fig. 2) [39-41]. In humans, N-desmethyltamoxifen is the primary metabolite followed by endoxifen and then 4-hydroxytamoxifen. These metabolites are potent anti-estrogens and are used to understand the tamoxifen’s mechanism of action [42,43]. Tamoxifen’s pharmacological profile indicates that it is a prodrug, and its anticancer activity occurs via its active metabolite, 4-hydroxy tamoxifen (8) and its desmethyl analogue endoxifen (10), which are generated by the action of hepatic CYP 2D6 and CYP3A4/3A5 isozymes on tamoxifen after hydroxylation followed by N-demethylation [44-45]. It has been established that patients with variant forms of the gene CYP 2D6 do not receive therapeutic benefits from tamoxifen administration or even suffer relapses because of slow tamoxifen prodrug metabolism into its active metabolites [46-48].

Several interesting facts associated with tamoxifen and its metabolites made it a pioneering agent for initiating new therapeutic investigation, and its pharmacogenomics is playing a significant role in redefining health care. In recent years, some of the tamoxifen analogues; for example, droloxifene (3), idoxifene (4), lasofoxifene (5), toremifene (6) and ospemifene (7) (Fig. 1) have been studied extensively in clinical trials [49]. Literature reports indicate
that continuous efforts are being made by researchers worldwide to identify novel tamoxifen derivatives for breast cancer and other therapeutic targets. This review is an effort to provide readers with information about recent and continuing development in this research area.

2. Therapeutic potential of tamoxifen derivatives

In recent years the biological activity of tamoxifen has been explored extensively in different therapeutic targets, and the results are very encouraging. In addition to breast cancer, tamoxifen has been used to treat infertility, gynecomastia, retroperitoneal fibrosis, and idiopathic sclerosing mesenteritis [50–52] and exerts beneficial effects on osteoporosis and reduces the incidence of cardiovascular diseases [53–55]. This drug has also been studied to treat Riedel's thyroiditis, bipolar disorder, and McCune-Albright syndrome [56–58]. Tamoxifen has a wide range of activities, including antifungal, antioxidant, and antiviral (especially antiviral hepatitis C virus activity) activities, antiangiogenesis properties, induction of intracellular calcium release, stimulation of transforming growth factor beta secretion, alteration of cellular membrane properties, and apoptosis induction [59–66]. Tamoxifen has been shown to target a number of proteins, including calmodulin, protein kinase C, phospholipase C, phosphoinositide kinase, P-glycoprotein, and swell-induced chloride channels [67]. Tamoxifen has exhibited very promising anticancer activity against different breast and other tumor cell lines (Fig. 3) [71,81,112,119,122,127]. The discovery of tamoxifen as an efficient therapeutic agent facilitated the synthesis of various analogues through chemical structure modification of tamoxifen and its metabolites. The reported tamoxifen derivatives exhibited encouraging biological activities and provided support in understanding tamoxifen's mechanism of action. Researchers around the world modified tamoxifen's chemical structure by substituting amino alkyl and ethyl chains with several groups and also by introducing substituents on phenyl rings. Moreover, in recent years, some reports have highlighted the importance of incorporation of the additional methylene or heteroaromatic group in the tamoxifen skeleton in addition to its conjugation with important moieties. In this review, we have made efforts to categorize tamoxifen derivatives on the basis of their structural modifications and further discussed their pharmaceutical significance. Moreover, tamoxifen metabolites analogues are also taken into consideration.

2.1. Tamoxifen derivatives with modification on aminoalkyl chain

The presence of a basic aminoalkyl chain on tamoxifen plays a major role in its antiestrogenic activity. Replacement of one N-methyl group of aminoalkyl side chain by a N-(2,2,2-trifluoroethyl) group has a detrimental effect, and the compound loses its potency to inhibit the growth of the ER+, MCF-7 cells [68]. In 2006, Agouridas et al. reduces the basicity of side chain by synthesizing a series of fluorinated derivatives of tamoxifen and studying their effects on their activities [69]. In addition to examining the effects of the substituents' chain lengths, the corresponding non-fluorinated analogues were also prepared. Analysis of the prepared compound’s binding affinity revealed that the fluorinated compounds’ binding affinity (11a–c) (Fig. 4) was one order of magnitude less than tamoxifen (RBA -0.2%–0.3%), and the non-fluorinated analogues (11d–f) (Fig. 4) were as effective as tamoxifen. Hence it was proposed that decreasing the basicity by replacing the methyl group of amino alkyl chain with a fluorinated moiety decreases tamoxifen analogues’ capacity to bind to the ligand binding pocket in addition to reducing the ER-mediated antagonistic properties. The fluorinated compounds displayed diminished ability to inhibit growth, stabilize ER, and to modulate ethylene response factor (ERF) and activator protein (AP-1) transcriptional activities. In addition, enhancement of agonistic activity on growth was also observed. The efforts to limit metabolic alterations of the tamoxifen’s amino alkyl side chain were not successful. It was suggested that additional thoughts and efforts are needed to find an atom or a group as a substitute for the nitrogen atom.

2.2. Tamoxifen derivatives with modification on amino alkyl chain and phenyl rings

In 2007, Shiina et al. reported the short step synthesis and cytotoxicity activity of pseudo-symmetrical tamoxifen derivatives bearing aminoalkyl chains on two phenyl rings [70]. The three pseudo-symmetrical tamoxifen derivatives (12a–c) (Fig. 4) were synthesized using a three component coupling reaction between the aromatic aldehyde, cinnamyltrimethylsilane, and aromatic nucleophiles with a Lewis acid catalyst. The antitumor activity of these compounds was determined against human promyelocytic...
leukemia (HL-60) cancer cells using MTT assay in which the efficiency of pseudo-symmetrical tamoxifen derivatives were determined by measuring their capacity to decrease cell viability. It was observed that compounds 12a and b, bearing pyrrolidine and piperidine side chain respectively, showed better cytotoxic activities and induced apoptosis in the HL-60 cancer cells. In contrast, compound 12c with a morpholine side chain was inactive when used in the same cell line. 12a and b effectively reduced cell viability in a dose-dependent manner. 12a, at final concentrations of 5, 7.5, and 10 \( \mu \text{M} \) and a 6-h incubation period inhibited cell viability by >90%. Similarly, compound 12b at final concentrations of 7.5 and 10 \( \mu \text{M} \) and a 6-h incubation period inhibited cell viability by >80%. In order to further confirm that 12a- or b-induced cell death was caused by apoptosis or necrosis, agarose gel electrophoresis for DNA cleavage was performed with HL-60 cells and 9 \( \mu \text{M} \) final concentration of the tested compound. The result was positive, and DNA fragmentation was observed, which suggested that the pseudo-symmetrical tamoxifen derivatives a12 and b induced cell death through apoptosis.

Christodoulou et al. (2013) designed and synthesized novel derivatives of tamoxifen by substituting tamoxifen’s aminoalkyl group with an amide side chain [71]. Although in all the compound of the series triarylethylene skeleton of tamoxifen having hydroxyl group in position 4 of the phenyl moiety was sustained. The derivatives were synthesized using McMurry coupling reaction and the characterization and structural assignment of \( \text{E, Z} \) isomers were assisted by 2D-NOESY experiments. The compounds’ \textit{in vitro} anti-proliferative activities were performed on three different cell lines: 1.) human breast cancer cell line (MCF-7), which overexpress the estrogen receptor; 2.) HeLa, an estrogen-independent human tumor cell line (cervix adenocarcinoma); and 3.) MSTO-211H, another estrogen-independent tumor cell line (biphasic mesothelioma). With respect to tamoxifen, most of the compounds in the series showed comparable or even higher antiproliferative activity on estrogen sensitive MCF-7 cells, whereas when compared with 4-hydroxytamoxifen, the compounds in the series were less effective. Also, the \( \text{Z} \) isomers exhibited slightly more activity toward MCF-7 cell lines compared to \( \text{E} \) isomers. With respect to antiproliferative activity on MCF-7 cell lines, the most active compound of the series was 13 (Fig. 4), which showed a GI\(_{50} = 2.9 \mu \text{M}\) that is 4 times higher than the reference drug, tamoxifen (GI\(_{50} = 12.0 \mu \text{M}\)).

As expected, tamoxifen’s antiproliferative effects on two estrogen-independent cell lines (HeLa and MSTO-211H) was much reduced (GI\(_{50} = 32.6 \mu \text{M}\) and 23.3 \( \mu \text{M} \), respectively). In comparison, except for 13 (HeLa, GI\(_{50} = 7.4 \mu \text{M}\) and MSTO-211H, GI\(_{50} = 8.4 \mu \text{M}\) most of the derivatives exhibited GI\(_{50}\) values for HeLa and MSTO-211H similar to the values obtained for MCF-7 cells. These results suggested that novel tamoxifen derivatives’ mechanisms of action with
respect to antiproliferative activity were independent from those actions on the estrogen receptor. To further study the possible molecular targets responsible for the cytotoxicity, the effects on relaxation activities of DNA topoisomerase I and II were assayed. These novel derivatives were able to inhibit topoisomerase II-mediated relaxation activity; therefore, it was suggested that potential intracellular targets could be nuclear enzymes.

Furthermore, Ridaifen-B (12a) (Fig. 4), a tamoxifen derivative, has shown higher potency than tamoxifen with respect to inducing ER-cell apoptosis via mitochondria membrane potential perturbation [72]. Thus, Hasegawa et al. (2014), using a series of tamoxifen derivatives that consisted of different Ridaifen forms, identified nonpeptide and noncovalent inhibitors of the human 20S proteasome [73]. The most active derivative was 14 (Fig. 4), which, at submicromolar levels (IC50 = 0.64, 0.34, and 0.43 μM) inhibited the three different enzymatic activities (CT-L, T-L and PGPH, respectively) of the 20S proteasome. A series of 14 analogues were prepared and then examined in order to study structure-activity relationships. The smallest analogue of 14 was compound 15 (Fig. 4), which was found to inhibit proteasome activity with potency similar to 14. These derivatives induced apoptotic cell death via the proteasome inhibition within the cells. Kinetic analyses of the inhibition and docking simulations suggested that the analogue of 14 interacted with the protease subunit in a different manner. The selective estrogen receptor down regulators (SERDs) are a class of antagonists that not only inhibit the binding of estrogens such as 17β-estradiol (E2) to the ER but also can induce rapid ER down-regulation [74,75]. They have no agonistic activity in many tissues and have been considered useful for ER+ or tamoxifen-resistant breast cancer cells [76]. Shoda et al. (2014) designed and synthesized tamoxifen derivatives by introducing a long alkyl chain (such as hexyl, dodecyl, and octadecyl) on 4-hydroxytamoxifen’s amine moiety and then evaluated its ability to facilitate ER degradation in MCF-7 cells and also examine its binding affinity for ER [77]. It was expected that a long alkyl chain on the amine moiety may destabilize the ER by protruding from the ligand binding pocket and thus inhibiting helix 12 interactions and coactivator binding. These results suggested that the presence of a secondary amine is essential for ERz down-regulation. The most promising compound in the series was 16 (Fig. 4), which, at 10 μM caused a reduction in the ERz protein levels in cells that were treated with this compound. In addition, a decrease in the ERz level in MCF-7 cells was observed in a concentration-dependent manner at doses of compound 16 ranging from 1 to 30 μM after a 6-h incubation period. It was observed that compound 16-induced reduction in ERz was caused by cellular proteosomal degradation.

Later, in order to obtain more potent SERDs and to explain the mechanism of ER down-regulation, six new tamoxifen derivatives that had various length and terminal groups (octyl, decyl, tetradecyl, hexadecyl, 10-hydroxydecdexyl, and 10-fluorodecyl) of the long alkyl side chain were synthesized [78]. Western blotting results showed that C10-bearing decyl group on the amine moiety of 4-OHT were most potent among the compounds having simple alkyl chains on the amine moiety; thus, alkyl chain length appears to play a significant role in the ER down-regulation. ER binding affinity studies revealed that compound 17a’s binding affinity (Fig. 4) for ERz (IC50 = 3.6 nM) was better than 4-hydroxy tamoxifen (IC50 = 5.6 nM). The introduction of the fluoro group on the alkyl chain terminus (17b) maintained high ERz binding (IC50 = 3.4 nM) and increased the potency of SERD activity. However, introduction of hydrophilic hydroxyl group on alkyl chain terminus (17c) decreased binding affinity for ERz (IC50 = 210 nM) in addition to reducing the ability to down-regulate the ERz protein levels.

It has been reported that tamoxifen can induce apoptosis in both ERz-positive and ERβ-negative breast cancer cells via different pathways; for example, pathways involving production of oxidative stress, induction of mitochondrial permeability transition, ceramide generation or changes in cell membrane fluidity may be involved [79]. Tamoxifen was also shown to inhibit DNA topoisomerases and be taken up by hepatic mitochondria in order to attain high concentrations with the purpose of inhibiting both β-oxidation and respiration [71,80]. Based on these results Christodoulou et al. (2016) synthesized a few tamoxifen analogues that showed significant antiproliferative activity on both estrogen-dependent (MCF-7) and -independent (HeLa) human tumor cell lines. This revealed involvement of a molecular target different from the ER [81]. The most promising compounds of the series were syn isomers with an isobutyramido moiety as side chain. These compounds exhibited 4-fold more potent cytotoxicity than the reference drug on both estrogen-dependent and -independent human cell lines. Additionally, these compounds displayed the ability to inhibit topoisomerase II-mediated relaxation activity of supercoiled pBR322 DNA. Further, a continuing novel series of tamoxifen analogues having triaryl skeletons substituted with the isobutyramide moiety was prepared. The antiproliferative activity of these compounds was evaluated on two human tumor cell lines (MCF-7 and HeLa) and two human ovarian cancer cell lines (A2780 and OVCARS). These compounds’ biological activities suggested that the presence of an OH group or Cl moieties in the aromatic ring B favors cytotoxic activity, whereas a methoxy or hydroxyl substituent in the aromatic ring A has a detrimental effect on cytotoxic activity. Most of the compounds were more active in MCF-7 and HeLa cell lines compared to A2780 and OVCARS cell lines. Compound 18 (Fig. 4) showed the lowest GI50 values in MCF-7 and HeLa cell lines (GI50 = 6.6 μM and 2.2 μM, respectively). Additional studies with 18 suggested that it was able to induce apoptosis and that topoisomerase II was a possible intracellular target.

2.3. Tamoxifen derivatives with modification on amino alkyl and ethyl chain

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat rheumatoid arthritis and osteoarthritis, and these agents mostly act through the cyclooxygenase (COX) inhibitory pathway. Several reports have suggested that selective COX-2 inhibitors are associated with fewer side effects compared to nonselective one [82,83]. Selective COX-2 inhibitors are also useful in the treatment of a wide variety of cancer and neurodegenerative disorders [84]. In 2004, Uddin et al. designed and synthesized acyclic triaryl olefinic compounds, which were structurally similar to tamoxifen, as selective COX-2 inhibitors [85,86]. The presence of identical C-1 phenyl substituents excluded the possibility of (E)- and (Z)-isomers. The most active compound in this series was 1,1-diphenyl-2-(4-methylsulfonylphenyl)hex-1-ene (19) (Fig. 5), which exhibited high potency (IC50 = 0.014 μM) and selective COX-2 inhibitory activity (selectivity index >7142).

In the literature, sulfoximines, monoaza sulfonyl analogues, are considered potentially useful groups for drug development [87–89]. Considering the significance of triaryl alkenes and sulfoximines in medicinal chemistry, Chen et al. (2012) used sulfonyl-substituted triaryl olefin 19 as a starting point to synthesized sulfoximine-based analogues and 20 and 21 (Fig. 5) [90]. Initially, the COX inhibitory activity of the synthesized compounds was checked; however, the compounds showed very low COX inhibitory activity. Furthermore, sulfoximines (20 and 21) estrogen binding affinities were evaluated using human recombinant enzymes, and sulfone 19 was used as a reference compound. Sulfone 19 was selective and moderately active for ER (77%) whereas sulfoximines (20 and 21) were basically ERα and ERβ unselective and exhibited
almost equal affinity for both ERs. Compound 21a (10 μM) showed maximum inhibition of up to 91% for ER α and 80% for ER β.

Literature reports have recommended that the tamoxifen’s triarylethylene framework acts as an estrogen agonist, and the two alkyl chains attached to it are responsible for full or partial antagonist behavior. Tamoxifen acts as a partial anti-estrogen agent after alkyl chain lengthening, and the presence of alkyl chains of different lengths on its amine moiety increase its antagonist effect in MCF-7 cells. Furthermore replacement of chloroethyl by an aminoethyl group in oospemifene (7), a triaryl ethylene SERM, resulted in substantial improvement in its anti-breast cancer activity [91–93]. In view of all of these factors, Kaur et al. (2016) designed and synthesized a new series of triarylethylenes by introducing polar amino-/amidoethyl groups instead of chloroethyl groups and replacing O-dimethylaminoethyl chains with short O-methyl chains [94]. To further examine the effect of structural modifications on tamoxifen, these compounds were evaluated for their anti-breast cancer activities against MCF-7 (ER+) and MDA-MB-231 (ER-) cell lines. Two forms of compound 22 (Fig. 5) (MCF-7 and MDA-MB-231 IC50 = 16.9 and 11.4 μM, respectively) and 23a (Fig. 5) (MCF-7 and MDA-MB-231; IC50 = 12.1 and 12.2 μM, respectively) showed promising activity against both the cell lines whereas compound 23b (Fig. 5) was selectively active only against MDA-MB-231 (MCF-7, IC50 ≤ 50 μM; MDA-MB-231, IC50 = 11.5 μM). Structure activity relationship studies suggested that the replacement of longer O-dimethylaminoethyl chains with O-methyl chains did not have any significant effects on its activities, whereas the introduction of an amino or oxalamido substitution on O-methyl analogues increased the potency in addition to making these analogues more effective against both ER+ and ER-cell lines. Furthermore, western blotting and scratch assays were performed to evaluate two parameters: 1.) the expression of proteins associated with adhesion, migration and metastasis and 2.) migration of human breast cancer MDA-MB-231 (ER-) cells. In both studies, compound 22 was most effective and it also showed anti-metastasis properties by suppressing wound healing and invasion. Experimental toxicity against MCF-7 cell lines was supported through in silico molecular docking studies. Representative compounds 22 and 23a exhibited good binding affinity with ERs.

2.4. Tamoxifen derivatives with modification on ethyl chain and phenyl rings

In the human genome, orphan estrogen-related receptors exist as three subtypes (specifically ERRα, ERRβ and ERRγ) [95]. The sequencing of these three orphan nuclear receptors sequencing is similar to classic estrogen receptors (ERα and ERβ), but they do not bind to estradiol or other related steroidal estrogens. The fasting-induced cofactor PGC-1α has been reported as an endogenous protein ligand for the ERRs [96–98]. ERRγ is expressed in the spinal cord and CNS, and it has been identified that the potent estrogen receptor antagonist 4-hydroxytamoxifen acts as an inverse agonist of ERRγ [99]. With the aim of developing ERRγ inverse agonist with selectivity against the classical estrogen receptors, Chao et al. (2006) selected 4-hydroxytamoxifen for further modifications and studies [100]. 4-hydroxytamoxifen analogues with extended ethyl side chain and polar functionality for ERRγ interactions and unfavorable interactions with ERα were designed and synthesized. The binding affinities of these compounds toward ERRγ and ERα suggested that analogues with hydroxalkyl groups were more selective in binding to ERRγ when compared with that of 4-hydroxytamoxifen. With analogue 24 (Fig. 5), a 25-fold improvement in binding selectivity for ERRγ over ERα was observed (ERRγ: IC50 = 0.079 μM; ERα, IC50 = 0.32 μM).

2.5. Tamoxifen derivatives with modification on phenyl rings, amino alkyl and ethyl chains

The literature indicates that the presence of tamoxifen’s aminaloxygen group is essential for its receptor binding affinity, and significant reductions in binding interactions were observed with a decrease in protonated amino groups’ basicity, whereas ethyl group replacement with a methyl group in tamoxifen showed no change in antiproliferative activity [101–103]. These results motivated Abdellatif et al. (2013) to design and synthesize tamoxifen analogues by replacing the dimethyl amino moiety in tamoxifen with secondary amines such as piperidino, piperazino, and/or N-methylpiperazino [104]. Additionally, the ethyl group was replaced by methyl group and the para position of phenyl ring was substituted
with a fluorine atom. The antiproliferative activity of these compounds was examined using an MTT assay for ER-positive and ER-negative cell lines (MCF-7 and MDA-MB-231 cell lines respectively). The most active compounds were 25a (Fig. 6) (MCF-7 and T47D-MB-231; IC_{50} = 6.75 and 10.53 μM, respectively) and 25b (Fig. 6) (MCF-7 and MDA-MB-231; IC_{50} = 5.58 and 13.04 μM, respectively), which showed better activity compared to tamoxifen (MCF-7 and MDA-MB-231; IC_{50} = 27.96 and 64.85 μM, respectively) in both the cell lines; however, the rest of the compounds showed activities similar to tamoxifen. Docking studies revealed that the synthesized compounds have a low docking score energy with ERα. In addition, compounds 25a and b (the most active ones) showed hydrogen bond interactions with the Asp-831 amino acid. Compounds 25a and b were capable of inhibiting MDA-MB-231 proliferation and indicated that the increased anticancer activities were ER-independent. Further tests were performed with 25a and b in order to explain their ability to induce ER-independent cell death. The results revealed that both the compounds were highly capable of triggering classic caspase-dependent apoptosis and exhibited high caspase 3/7 activities in MCF-7 treated cells compared to tamoxifen. The most active compound was examined using an MTT assay for ER-positive and ER-negative cell lines (MCF-7 and MDA-MB-231 cell lines respectively). Most of the compounds showed similar or better activities than tamoxifen, and the most active compound in the MCF-7 cell line was compound 27 (Fig. 6) (IC_{50} = 3.6 μM). The 4-hydroxytamoxifen is one of the main metabolite of tamoxifen and is well known to have better activity than the parent compound [113,114]. Similarly, in this study, the 4-hydroxy derivatives were more potent than their parent compounds. The alkene geometry plays a very important role in tamoxifen activity; subsequently 2-tamoxifen acts as an antagonist, whereas E-tamoxifen behaves as an agonist. In contrast, this series of fluorinated compound exhibited similar activities for both geometrical isomers, and it was suggested that this behavior might be due to the compounds' in vitro isomerization.

Furthermore, to identify the structural features of tamoxifen that confer selective binding properties to the ER relative to other targets such as protein kinase C (PKC), extensive structure activity relationship studies have been done with the tamoxifen framework. Tamoxifen's high affinity for estrogen and low affinity for PKC compromise its utility to selectively target PKC for breast disorders. Carpenter et al. (2016) used tamoxifen's triphenylethylenic core as a framework to design and synthesize molecules with increased affinity for PKC and decreased affinity for the ERs [115]. Based on the previous study of Bigon et al., compound 28a (Fig. 6), bearing a diethyl amino side chain, was selected as the lead compound for further modification [116]. Compound 28a exhibited reasonable PKC inhibition (12% at 3 μM) and lower ER binding affinity (IC_{50} = 10 000 nM) relative to tamoxifen (PKC inhibition = 27% at 3 μM and ER binding affinity, IC_{50} = 222 nM). A series of novel triarylacrylonitrile analogues were synthesized and screened for PKC inhibition and ER binding affinity. The most potent compound of the series was 28b, containing a more basic (4-methyl) anilino-1-yl)ethoxy side chain, significantly inhibited PKC at a concentration of 3 μM (83%) and caused a decrease in ER binding affinity (IC_{50} = 10 000 nM) compared to tamoxifen.

Cytochrome P450 (CYP450), mainly CYP2D6 and CYP3A4 enzymes, are involved in tamoxifen metabolism and activation to the more active 4-hydroxy tamoxifen (8) and endoxifen (10). High variations in tamoxifen's clinical outcomes can be observed as a result of genetic polymorphisms in the CYP2D6 genes [117,118]. In 2016, Ahmed et al. designed and synthesized tamoxifen analogues by retaining tamoxifen's pharmacophoric features and following possible metabolic pathways that do not involve the CYP2D6 enzyme [119]. These analogues were evaluated for their antiproliferative activity on MCF-7 breast cancer cell lines and binding affinity for ER-α and ER-β receptors. All of the compounds showed better antiproliferative activity than tamoxifen on MCF-7 cells. The most promising compound was 29 (Fig. 6), with a G_{50} = 0.005 μM and a 1000 times more potency than tamoxifen (G_{50} = 1.58 μM). In addition, after incubation of compound 29 in human liver microsomes (HLM) and human hepatocytes (HHEP), the active hydroxy metabolite was detected; this suggests other enzymes' involvement in its metabolism.

Combretastatin A-4 (CA-4), a natural product having an aryl-ethylene moiety, is a potent tubulin polymerization inhibitor (IC_{50} = 1.2 μM) in addition to possessing strong cytotoxic activity against selected human cell lines such as DU-145 prostate cancer cells (G_{50} = 2 nM) [120,121]. In comparison to CA-4, tamoxifen has no significant effects on tubulin polymerization (IC_{50} > 40 μM). Consequently, Tanpure et al. (2009) designed and synthesized tetra-substituted alkenes by combining structural and electronic components of tamoxifen and combretastatin A-4 using McMurry
coupling [122]. The compounds were evaluated for their inhibition of tubulin polymerization and cell growth in selected human cancer cell lines. Altogether the compounds were less potent than CA-4, and none of them were able to significantly inhibit tubulin assembly. Of all the series, compound 30 (Fig. 6) was more cytotoxic than tamoxifen against the three cell lines, especially against the human ovarian cancer cell line SK-OV-3 (G150 = 0.6 µM). Similar to tamoxifen, compound 30 was not able to inhibit tubulin assembly (IC50 > 40 µM); therefore, it was assumed that compound 30’s cytotoxicity occurred via different mechanisms.

### 2.6. Flexible tamoxifen derivatives

Meegan and coworkers studied several flexible and non-isomerisable estrogen receptor modulator analogues of tamoxifen, and the most potent lead compound was identified as 31 (Fig. 7), which contained an additional methylene group positioned between the aryl ring C and vinylic carbon and had an IC50 12.5 µM against MCF-7 cell line [123,124]. Furthermore to improve tamoxifen analogues’ anti-proliferative and receptor binding activities, a second generation derivatives of compound 31 was synthesized by introducing a hydroxyl group on ring B [125]. The effects of halogens and other oxygen containing species (such as esters and carbamates) on the phenyl ring in addition to the effects of different amino alkyl chains were examined. Most of the second generation forms of compound 31 exhibited high anti-proliferative activity against the MCF-7 human breast cancer cell lines. The cytotoxic assessment results suggested that the compounds exhibited low cytotoxicity, indicating their mode of action is cytostatic rather than cytotoxic. The most active compound was 32 (Fig. 7), which showed high ER binding affinity (IC50 = 20 nM) with up to 12 fold ERα/β selectivity. The compound also displayed anti-estrogenic effects at 40 nM with little estrogenic stimulation when evaluated in the Ishikawa cell lines and also promoted apoptosis in MCF-7 cells in a FACS based assay. The docking study of compound 32 was performed using the crystal structure obtained from the cocrystallization of ERα with 4-hydroxysteroidinamide as found in the PDB database (PDB ID 3ERT) [126]. The results suggest that compound 32 binds in an anti-estrogenic manner with some modifications in its benzylic ring orientation.

The genetic polymorphisms in CYP2D6 are considered responsible for variation in tamoxifen’s clinical outcomes. The polymorphism may result in the formation of inactive proteins devoid of enzymatic activity or may lead to an enzyme with reduced activity. Bearing in mind the anti-proliferative activity of flexible tamoxifen analogues, in 2016, Elghazawy et al. synthesized a series of flexible tamoxifen analogues to overcome the genetic polymorphism of the CYP2D6 enzymes [127]. The site for metabolic para-hydroxylation was blocked by introducing either a hydroxyl or ester group, and flexibility to the rigid triphenylethylene backbone was provided by introducing a benzylc methylene spacer between the phenyl ring and ethylene group. Additionally, several analogues of tamoxifen bearing dimethylaminoethoxy, pyrrolidinylethoxy, or piperidinylethoxy side chains were synthesized, and the effects of cyclization, size of the cyclic structure, and the effects of altering the nitrogen’s basicity have been studied. The tamoxifen analogues were evaluated for their anti-proliferative activity on MCF-7 cell lines and their binding affinities for ERα and ERβ. All of the tamoxifen analogues showed better anti-proliferative activity than tamoxifen, and the most active compound 32 contained pyrroldinylethoxy side chain (Fig. 7) exhibiting IC50 < 0.25 µM. Compound 32 also exhibited 80-times more ERα binding than tamoxifen and 900 times more selectivity towards ERα than tamoxifen. The mode of compound 32’s binding with ERα was examined with a computational docking study using crystal structure obtained from the co-crystallization of ERα with 4-OHTAM [126]. As anticipated, the results showed that introduction of the additional methylene group allow the compound to easily adopt the required arrangement for binding in an established anti-estrogenic mode.

### 2.7. Tamoxifen derivatives with heteroaromatic groups

Considering the ER binding affinity and anti-estrogenic activity of heteroatom containing tamoxifen analogues [128,129], Wenckens et al. (2003) designed and synthesized a series of tamoxifen analogues by replacing the 1Z-alkoxyphenyl group by N-alkoxyopyrazole and by introducing the functionalized phenyl or heteroaromatic groups at the 2Z-position of tamoxifen [130]. Also, a few analogues of 4-hydroxytamoxifen were prepared in which the 1E-4-hydroxyphenyl group was replaced with a 1-hydroxypyrazol-4-yl group. The binding affinity of the synthesized N-alkoxypyrazole analogues to the estrogen receptor (ERα) were determined and compared with tamoxifen. Substantial differences in binding affinities to the ERα were observed between the 4’ and 5’ pyrazolyl analogues. Most of the compounds of the 4’pyrazolyl series exhibited good binding to the ERα and were comparable to tamoxifen (IC50 = 0.1 µM). The compounds’ cytostatic properties were examined in the MCF-7 cell lines and showed high affinity for ERα. The activity results suggest that replacement of the phenoxo group in tamoxifen with 1-pyrazolyl group does not have significant effects on its anti-estrogenic activity. Compound 33 (Fig. 8), which is a close tamoxifen analogue, showed growth inhibition.

![Fig. 7. Flexible tamoxifen derivatives (31 and 32).](image1)

![Fig. 8. Tamoxifen derivatives with heteroaromatic groups (33–36).](image2)
(IC₅₀ = 3 μM) similar to tamoxifen (IC₅₀ = 2.7 μM) on MCF-7 cell lines. Moreover, the introduction of substituents in the 2Z- or 1Z-phenyl group or thiienyl group does not display significant changes in the potency. The most potent compound of the series was compound 34 (Fig. 8), which was unable to isomerize around the double bond and inhibited the growth of the MCF-7 cell line at IC₅₀ = 1.0 μM.

Taking into consideration the abundance of the structural component quinone and diene in several cytotoxic agents [131–134], Srivastava and his co-workers (2015) designed and synthesized a series of constrained tamoxifen look alikes containing the spirodienone moiety [135]. The series was synthesized using iodine-catalyzed ipso-cyclization followed by Suzuki coupling. The molecular docking results of the series suggest that these compounds fit properly in the binding pocket and have similar binding mode in ER binding site as that of the co-crystallized ligand 4-hydroxytamoxifen. The compound’s series was also evaluated in vitro against ER+ MCF-7 and ER-, MDA-MB-231 breast cancer cell lines. Most of the compounds were active against MCF-7 cells with IC₅₀ values < 6.5 μM. Compound 35a (Fig. 8), containing a 4-hydroxy substituent on the phenyl ring, showed maximum activity against both cell lines (MCF-7 and MDA-MB-231; IC₅₀ = 5.76 and 8.85 μM, respectively), and compound 35b (Fig. 8), containing a 4-methoxy group on the phenyl ring, was most selective against MCF-7 cells (MCF-7 and MDA-MB-231; IC₅₀ = 5.86 and 64.0 μM, respectively).

Pharmaceutical limitations associated with tamoxifen, existing as two geometrical stereoisomers with opposite actions, leads to the development of nonisomerizable antestrogens such as nafoxidine and ring-fused analogues such as benzocycloheptene, benzoxepine, and benzothiepins [136–137]. The problem of isomerization was solved with the discovery of these non-isomerizable compounds; however, these compounds were not potent for antiapoptotic or antiestrogenic activity. Consequently, Ansari et al. (2015) designed and synthesized a novel series of substituted dibenzo[b,f]thiepins and dibenzosepi[b,f]oxepines as anti-breast cancer agents [138]. The compounds were tamoxifen analogues and contained planar tricyclic cores with pendant phenyl rings as attachments. The designed antiestrogenic conformation was achieved by the presence of basic tert-aminoaalkoxy group on the phenyl ring with perpendicular orientation. The synthesized compounds were evaluated on ER+ and ER-breast cancer cell lines and the results showed that most of the compounds possessed promising in vitro antiapoptrophic activity. The dibenzo[b,f]thiepins analogues with the sulfur atom were more active as anti-breast cancer agents compared to oxygen containing dibenzo[b,f]oxepines analogues. The most active compound of the series was 36 (Fig. 8) that inhibited both the breast cancer cell lines in the micromolar range (MCF-7 and MDA-MB-231; IC₅₀ = 1.33 and 5 μM, respectively). Also, it lacked any cytotoxic effects at 50 μM on the normal human embryonic kidney (HEK-293) cells, which was much better than tamoxifen at 18 μM. Further analysis of compound 36 on cell cycle distribution and apoptosis of MCF-7 cells followed by an LDH release assay suggested that 36 inhibited cellular proliferation via G0/G1 arrest in MCF-7 cells and was primarily due to apoptosis, not necrosis. As anticipated, molecular docking studies showed better binding of compound 36 with estrogen receptors in comparison to 4-hydroxytamoxifen binding to the same receptors.

2.8. Tamoxifen conjugates

Introduction of the organometallic substituent on the tamoxifen skeleton and its effects on cytotoxicity were first studied by the Top and Jaouen group. Initially, some ferrocenyl derivatives of hydroxytamoxifen (37) (Fig. 9) were prepared by substituting the β-phenyl ring of 4-hydroxytamoxifen with a ferrocenyl unit. The biological activity results of ferrocifen compounds (37) demonstrated that these compounds had strong antiproiferative effects on both hormone-dependent (ER+) and -independent (ER-) breast cancer cells [139,140]. Motivated by these results in 2007, the group synthesized compound 38 (Fig. 9), in which the amino alkyl chain of 4-hydroxytamoxifen was replaced by a stable and a lipophilic ferrocenyl group —OCH₂CO-[[(CH₃)₂C₅H₄]FeCp] [141]. The relative binding affinity test with estrogen receptors showed positive results for these compounds. As expected, the (Z) isomer of 38 was well recognized and showed good affinity for both the ERα and β estrogen receptor isoforms (13.9% and 12.8%, respectively), and the (E) isomer exhibited a drop in affinity with both isoforms (ERα and β [1.2% and 1.6%, respectively]). Next, to understand the binding affinities the molecular modeling on (E)-38 and (Z)-38 on the anti-estrogenic form of the estrogen receptor was investigated. The study suggested that carbonylferrocenyl group substitution has minimal effects on the interactions of ligand to the ERα, and the [(Z)-38]-cavity complex was found to be twice as stable as that of the [(E)-38]-cavity complex. The antiproliferative activity of both diastereomers was examined on hormone-dependent MCF-7 breast cancer cells and hormone-independent PC-3 prostate cancer cells. Both diastereomers 38 displayed the antiproliferative activity, with an average of 10.4 μM on MCF-7 cells and 8.9 μM on PC-3 cells, which is not as strong as that of the ferrocinens 37 (n = 3; IC₅₀ = 0.5 μM on MCF-7). It was anticipated that the antiproliferative activity of 38 could occur either through an anti-hormonal mechanism or was due to the cytotoxic character of the ferrocenyl group.

Subsequently, a series of cobaltifens, organometallic analogues of tamoxifen in which a phenyl ring has been replaced by an organo-cobalt sandwich moiety, was synthesized [142]. The presence of the organo-cobalt sandwich supports multiple functionalizations and allows modification of its electronic characters, redox properties, and steric parameters [143]. The compounds were screened for their ERz binding affinity and antiproliferative activities against hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cells. The ERz binding affinities for cobaltifens were quite low (below 1%) and significantly lower than ferrocinyl derivatives (RBA 10–15%). It was suggested that this could be because of the size of the organometallic cobalt units with four phenyl groups which is significantly more bulky than the unsubstituted cyclopentadienyl ring of ferrocene. Cellular proliferation results revealed that the dihydroxycobaltifens showed estrogenic activity against MCF-7 cells but not on MDA-MB-231 cells, whereas, the aminoketyl-hydroxycobaltifens were weakly cytotoxic toward both cell lines. Surprisingly the bis-(dimethylamino-ethoxy)cobaltifens such as 39a and b (Fig. 9) exhibited high cytotoxicity toward both cell lines, specifically MDA-MB-231 cells (IC₅₀ = 3.8 and 2.5 μM, respectively).

Later, considering the significance of ferrocenyl derivatives of hydroxytamoxifen for antiproliferative activity against both estrogen-responsive and estrogen-refractory breast cancer cells [144,145], a series of tetrasubstituted olefins bearing a ferrocenyl group were designed and synthesized [146]. Earlier reports indicated the antiproliferative studies were done only on breast and prostate cancer cell lines; hence to obtain a broader view, using the MITT test, the synthesized compounds were evaluated on four tumor types, including SF-295 (human glioblastoma), HCT-8 (human colon cancer), MDA-MB-435 (human melanoma), and HL-60 (human promyelocytic leukaemia). More than one third of the compounds exhibited IC₅₀ values < 2 μM for one or more cell lines. The most encouraging compound of the series was 40 (Fig. 9), which was a 2-ferrocenyl-1,1-diphenyl-but–1-ene that showed an interesting combination of growth inhibition and low hemolytic activity.

Fig. 9. Tamoxifen conjugates (37–45).
and was then selected for further examinations. This compound exhibited promising activities on SF-295 (IC50 = 1.0 μM), HCT-8 (IC50 = 0.9 μM), and HL-60 (IC50 = 1.04 μM) cell lines and moderate activity on the MDA-MB-231 breast cancer cell line (IC50 = 16 μM).

Selective targeting and delivery of gold nanoparticles functionalized with ligands of cell surface receptors overexpressed by malignant cells has been well documented [147–150]. Moreover, ER isoforms are located both intracellularly and on the cell membrane [151,152]. After considering these facts, Dreaden et al. (2009) synthesized gold nanoparticle tamoxifen analogues as selective and potent agents for breast cancer treatment [153]. The thiol(poly(ethylene glycol) (OEG) tamoxifen (PEG-SH-TAM) derivative was synthesized and subsequently conjugated with gold nanoparticle (AuNP). Tamoxifen–gold nanoparticle conjugate (TAM-PEG-SH-AuNP; 41) (Fig. 9) exhibited drug potency 2.7 times greater than free tamoxifen; this was due to the conjugate’s selective intracellular delivery to ER+ breast cancer cells, caused by both receptor- and ligand-dependence in vitro. These results suggest that plasma membrane-localized ERα may facilitate selective uptake and retention of this and other therapeutic nanoparticle conjugates. Following a similar synthetic approach Nelson et al. (2011) synthesized single walled carbon nano tube (SWCNT) tamoxifen conjugates (42) (Fig. 9) and characterized them by using different analytical techniques, including NMR [154]. It was anticipated that the conjugate comprising both SWCNT and tamoxifen linked by octa(ethylene glycol) (OEG) could be used in breast cancer treatment due to the advantages of SWCNTs in drug delivery systems and photothermal therapy in addition to tamoxifen’s recognition properties as a selective targeting agent and potent endocrine treatment drug.

One of the important strategies for understanding the mechanisms of estrogen signaling and the specific physiological responses associated with this signaling is to develop new tamoxifen-based chemical probes. Only a few examples of fluorescent tamoxifen probes have been reported in the literature [155,156]. The probes having direct attachment of dyes to tamoxifen or metabolite were having good binding affinity and selectivity nevertheless they were not helpful to solve the contentious membrane receptor problems. The tamoxifen probe developed specifically for membrane associated ER studies also found to have poor affinity and loss of specificity compared to parent compound. These results inspired Ho et al. (2016) to design and synthesize new selective tamoxifen-based fluorescent probes [157]. Tamoxifen was selected (instead of its metabolite 4-hydroxytamoxifen) to tether the fluorophore since it is commercially available and tamoxifen-based probes are known to have sufficient binding compared to the metabolite-based probes. Dye attachment was done on the basic alkylnoehoxy side chain with a short ethylene glycol linker. The BODIPY®FL fluorophore was selected for the study due to its well-characterized cell permeability and unusual cytoplasmic localization [158]. The cellular localization of the synthesized fluorescent BODIPY®FL ethylene glycol-linked tamoxifen conjugate (43) (Fig. 9) was visualized by fluorescent confocal microscopy in ER-positive MCF-7 and ER-negative MDA 231 breast cell lines. Results revealed that the BODIPY®FL conjugate (43) was internalized in the ER-positive cell via a receptor-mediated mechanism of uptake; however, no internalization of 43 was observed in ER-negative cells. Additional increases in concentrations of 43 exhibited no change in the degree of uptake or localization, and also no localization of 43 in the cytoplasm was observed.

In the literature, several reports have described the significance of the targeted drug design approach to be used to overcome multi drug resistance [159–163]. An anticancer agent can be attached to a compound known to accumulate in cancer cells, which increases its uptake into the cancerous cell in comparison to healthy cells. Following this approach Hawco et al. (2013) planned to conjugate prodigiosenes [164,165], a class of tripyrrolic compound with significant anticancer activity, to tumor selective ligands in order to deliver a drug to a specific site. Several ER ligands such as tamoxifen [166], fulvestrant [167], and hematoporphyrin [168] were selected to target ER positive breast cancers. The tripyrrolic skeleton was attached to the selected ligands via ester linkages with several hydrocarbon chain lengths [169]. The synthesized conjugates were screened for their in vivo biological activities on different cancer cell lines. The results revealed that the tamoxifen conjugates 44a and b (Fig. 9) were the most potent against MCF-7 cells with GI50 = 30 and 50 nM, respectively. The porphyrin conjugate was inactive, whereas the estrone conjugate exhibited moderate activity on both ER negative and positive cell lines. The structure activity relationship studies suggest that conjugates with shorter chain length conjugates, which are linked with 2 and 4 carbon atoms, are more active than the conjugates with a longer chain length (linker with 8 carbon atoms).

Ridaifen B (RID-B) 12a, a tamoxifen derivative, has been shown to be more active on cell proliferation compared to tamoxifen. RID-B is equally active on ER+ and ER-cell lines; its mode of action is different from the existing cancer drugs, including tamoxifen, suggesting an ER-independent mode of action [170]. In 2013, Sugawara and his co-workers investigated RID-B binding proteins via a T7 phage display screen and binding analysis with synthesized biotinylated RID-B derivative (Bio-RID-B) 45a (Fig. 9) [171]. The study identified Grb 10 interacting GYP protein 2 (GIGYF2) as an RID-B binding protein, which is involved in the PI3K/Akt signaling pathway and is up-regulated in breast cancer cells in which Akt is highly phosphorylated. RID-B binds directly to GIGYF2 and reduces Akt’s phosphorylation level, suggesting an ER-independent anti-cancer activity for RID-B’s mechanism of action. Subsequently, a biotinylated RID-G derivative (Bio-RID-G) 45b (Fig. 9) was synthesized and a chemical genetic approach was used to identify the target proteins of RID-G, another tamoxifen analogue containing a dimethyl amino alkyl chain on two phenyl rings and having high growth inhibitory activity against various cancer cell lines [172]. Using this approach, a phage display screen was combined with a statistical analysis using drug potency and gene expression profiles in thirty nine cancer cell lines. This approach assisted in understanding the physiological relevance of the direct association [173]. The study identified three proteins, calmodulin (CaM), heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1), and zinc finger protein 638 (ZNF638) as targets of RID-G as part of its growth inhibitory activity.

Tamoxifen and its main metabolite (4-hydroxytamoxifen) are known to form adducts with DNA, and in animal models hepatic toxicity has been observed in the presence of these two agents [174]. In 2014, Tajmir-Riahi and coworkers used chemical and molecular modeling approaches to examine the binding affinities of tamoxifen (1) and its metabolites for DNA with the aim of establishing the tamoxifen and its metabolites’ mechanism of binding to DNA [175]. The results suggested that tamoxifen and its metabolites bind to DNA via both hydrophobic and hydrophilic interactions. In the DNA duplex, tamoxifen and its metabolites showed different binding sites, and the order of binding was 4-hydroxytamoxifen (8) > tamoxifen (1) > endoxifen (10), indicating the formation of a more stable complex with 4-hydroxytamoxifen. Next, considering the fact that loading of tamoxifen and its metabolites with serum protein increases the solubility of the drug, improves its tissue-specific targeting, and facilitates constant release of the drug, a comparative study on the binding affinity of serum proteins with tamoxifen and its metabolites was performed [176]. In this study, both human and bovine
serum albumin (HSA and BSA, respectively) were used, and the results of multiple spectroscopic methods and docking studies were considered. The results revealed that tamoxifen and its metabolites bind serum proteins through hydrophobic, hydrophilic, and/or H-bonding interactions and that HAS conjugates were more stable than BSA conjugates. 4-hydroxytamoxifen forms a stronger bond than tamoxifen and endoxifen. Additionally, major drug conjugation-induced perturbations in serum protein conformation were observed, and 4-hydroxytamoxifen formed a stronger bond than tamoxifen and endoxifen. Later, encouraged by polyamidoamine (PAMAM) significance as drug delivery tools, either through physical interactions or chemical bonding, the loading efficacies of antitumor drugs (doxorubicin and tamoxifen) with PAMAM-G4 were reviewed, and correlations between drug interactions and polymer morphology were established [177]. The results indicated that hydrophilic, hydrophobic, and H-bonding contacts were responsible for drug-polymer conjugation and that doxorubicin formed more stable conjugates with PAMAM-G4 than did tamoxifen. The drug loading efficacy was 40%–50%, and PAMAM nanoparticles were observed to be efficient for both drugs in vitro transport.

2.9. Derivatives of tamoxifen metabolites

PKC is an important enzyme in cell signaling pathways. It is involved in numerous brain diseases such as Parkinson’s, Alzheimer’s, and bipolar diseases (BPD) in addition to substance abuse [184]. Preclinical and clinical studies with the relatively selective PKC inhibitory activity in vitro, predicted cellular PKC activity [182,183]. Tamoxifen is the only PKC inhibitor that can penetrate the blood brain barrier and inhibit cellular PKC activity [184]. Preclinical and clinical studies with the relatively selective PKC inhibitor Z-tamoxifen support the significance of PKC as a target for treatment of BPD [185]. BPD is a chronic, debilitating illness characterized by drastic swings in mood, energy, and functional ability, and it mostly affects the adult population [186]. Conversely, capriciousness was observed in tamoxifen bioavailability and function due to CYP2D6 genetic polymorphism, which extensively metabolizes tamoxifen into its active metabolites, 4-hydroxytamoxifen (8) and endoxifen (10). In 2010, Ali et al. synthesized endoxifen, an active metabolite of tamoxifen, studied its PKC inhibitory activity in vitro, and then compared it with the known PKC inhibitor, tamoxifen [187]. In comparison to tamoxifen, endoxifen significantly inhibited PKC in a concentration-dependent manner and was found to be four fold more potent. At the concentration of 0.2 mM, endoxifen exhibited 78% PKC inhibition, whereas the tamoxifen showed only 25%.

In postmenopausal women, third generation aromatase inhibitors (AIs) such as anastrozole, exemestane, and letrozole have mainly replaced tamoxifen as the preferred treatment for hormone receptor-positive breast cancer. Clinical studies have suggested that AIs are much more superior and effective than tamoxifen in the treatment of ER-positive breast cancer in postmenopausal women, and these drugs have demonstrated enhanced safety profiles, tolerability, and disease free survival rates [188–190]. On the other hand, the use of AIs as anti-breast cancer drugs also involves various side effects such as reduction of bone density, severe musculoskeletal pain, and increased frequency of cardiovascular and thromboembolic events due to the overall estrogen reduction [191–193]. Continuous efforts are being made by researchers worldwide to develop novel AIs with novel mechanisms that can cause fewer side effects. Incidentally, Cushman and coworkers (2013) thought to develop new breast cancer chemotherapeutic agents with dual aromatase inhibitory and estrogen receptor modulatory activities. Norendoxifen, a human metabolite of tamoxifen with high potency and selectivity as an AI [194], was selected as a lead compound for further development. It was expected that close structural similarity of norendoxifen with tamoxifen would preserve the dual aromatase inhibitory and ER modulatory activities. The aromatase inhibitory activity should inhibit tumor growth by blocking estrogen biosynthesis in the breast, and its ER modulatory activity should reduce the side effects in bones and other tissues caused by estrogen reduction. Initially (E)-norendoxifen (46a), (Z)-norendoxifen (46b), and (E, Z)-norendoxifen isomers (46c) (Fig. 10), were synthesized and their aromatase inhibitory and estrogen receptor affinities were determined [195]. Compound 46c displayed strong aromatase inhibitory activity with IC₅₀ = 102 nM and good binding affinity to both ERα and β with EC₅₀ = 27 nM and 35 nM, respectively. It was observed that 46a was a more potent aromatase inhibitor (IC₅₀ = 76.8 nM) than 46b (IC₅₀ = 1029 nM). In contrast, 46b displayed a higher binding affinity toward ERα (EC₅₀ = 17 nM) and β (EC₅₀ = 28 nM) than dihydroxytamoxifen (EC₅₀ = 59 nM and 79 nM, respectively).

Next, a series of structurally related norendoxifen analogues were designed by molecular modeling using a structure-based drug design approach. The designed analogues were synthesized and then pharmacologically evaluated. The most optimizing inhibitor against both aromatase and ER, improve the aromatase selectivity versus other cytochrome P450 enzymes, and to further explore the structure activity relationships [196]. Most of the analogues of the series exhibited promising aromatase inhibitory activities and ER binding affinities. The most potent compound was 4′-hydroxynorendoxifen (47) (Fig. 10) with high aromatase inhibitory potency (IC₅₀ = 45 nM) and ER binding affinity (ERα and β; IC₅₀ = 15 and 9.5 nM, respectively). In comparison to norendoxifen, analogue 47 was a more potent antagonist than norendoxifen of estradiol-stimulated progesterone receptor mRNA expression in MCF-7 cells. Also, selectivity of 47 for aromatase versus other cytochrome P450 enzyme was much better than norendoxifen (10).

Furthermore, in solution, E/Z isomerization was observed for most of the norendoxifen analogues that were similar to 4-hydroxytamoxifen and was possibly due to the presence of a phenolic hydroxyl group in one of the para positions [197]. An increase in the number of para phenolic hydroxyl groups increased the isomerization rate and was also dependent on the temperature and solvent [198]. E/Z isomerization can affect the synthesis of pure E and Z norendoxifen isomers in addition to influencing the accuracy of biological tests for pure E and Z isomers. Therefore, to overcome the problems associated with E/Z isomerization, a series of triphenylethylene bisphenol analogues was designed and synthesized for use as dual AI/SERM agents [199]. In these analogues, the possibility of E/Z isomerization was solved by eliminating norendoxifen’s aminoethoxy side chain. The hydrogen bond donor groups (such as hydroxyl or amino groups) were introduced at the meta or para positions of the phenyl ring as a substitute to the aminoethoxy side chain. In addition, iron-coordinating groups (such as nitrile, imidazole, or triazole groups) were used as substitutes for the ethyl group. The synthesized compounds were evaluated for their aromatase inhibitory activities, ER-α and -β binding affinities, and abilities to antagonize β-estradiol-stimulated transcriptional activity in MCF-7 human breast cancer cells. The biological activity results suggest that replacement of the ethyl group with an imidazole group was favorable for aromatase inhibitory activity and ER binding affinities. The most potent compound of the synthesized series was the imidazole-containing compound 48 in Fig. 10. Compound 48 displayed a very high aromatase inhibitory activity (IC₅₀ = 4.77 nM) in addition to ER binding affinities (ERα and β; EC₅₀ = 27 nM and 41 nM, respectively).
Later, to optimize the aromatase inhibition and ER binding affinity of norendoxifen analogues the structural features of a third generation AIs letrozole [200], which was a symmetrically substituted diphenylmethane fragment, was taken into consideration. The new series of norendoxifen [10] analogues were synthesized by eliminating the aminoethoxy side chain, substituting the para position of ‘A’ ring with a nitro/amino group and para position of ‘B’ and ‘C’ ring with hydroxyl or amino group [201]. The resulting analogues were devoid of geometrical isomerization and possessed hydrogen bond acceptors similar to letrozole. The biological activity results exhibited that the analogues bearing para- amino group in the ‘A’ ring of triphenylethylene scaffold was potent for aromatase inhibitor activity. The most promising compound of the series was 49 (Fig. 10) with significant aromatase inhibitory activity (IC₅₀ = 62.2 nM) and binding activity to both ERα and β (EC₅₀ = 72.1 and 70.8 nM, respectively). The structure activity relationships studies suggest that the presence of amino groups in the para position of ‘A’ and ‘B’ was favorable for aromatase inhibitory activity, whereas replacement of ethyl side chain with a methyl group had detrimental effects on aromatase inhibition and ER binding affinity. Replacement of the unsymmetrical diphenylmethylene substructure of norendoxifen with symmetrical diphenylmethylene substructure preserved the activity and eliminated the triphenylethylene’s E/Z isomerization. Additionally, in contrast to previous reports [202,203], it was observed that the aminoethoxy side chain in triphenylalkene derivatives was insignificant for both aromatase inhibitory and ER binding affinity.

3. Conclusion

In conclusion, we can certainly mention that tamoxifen is one of the important drug templates explored by researchers worldwide for cancer and other therapeutic targets. Tamoxifen has immensely decreased the death rates from breast cancer around the world, and patients are living longer, recurrence-free lives with less morbidity. Since the discovery of tamoxifen in the 1970s as a failed contraceptive to the promising breast cancer drug, the research on the tamoxifen template is still ongoing. It has been well-documented that tamoxifen tremendously supports the discovery of several significant compounds with promising biological activities, and in the future, discovery of more promising compounds is expected. This review article is an attempt to make available researchers the thorough progressions made in the last few years in the tamoxifen research domain and it’s our firm believe that it will support readers with their ongoing research. In the end, we would like to conclude with these lines: “A new dynasty gives over the ruling dynasty through perseverance and not by sudden action” (14th Century Arab Historian Ibn Khaldun) and “No advances occur in isolation; they build on the work of previous generations and by collegial interaction” (Father of Tamoxifen, V C Jordan).

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