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Review Article

The potent triarylethylene pharmacophore

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CONTENTS

Abstract

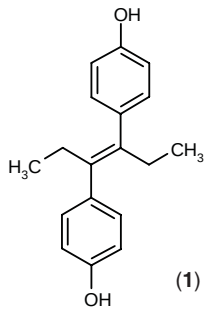
The triarylethylene (TAE) framework, a structural variant of the frank estrogen diethyl stilbestrol, forms the backbone of a number of nonsteroidal estrogen agonists as well as antagonists. Compounds with the TAE pharmacophore have been reported with promising biological activity in estrogen-dependent disorders such as breast cancer, osteoporosis, CNS/CVS and in fertility regulation. The structure-activity relationships of such compounds have delineated the structural requirement for estrogen agonist and antagonist activities. The use of such compounds for a particular area depends upon the ratio of these two activities. Some of the compounds have been found to be tissue-selective and are referred to as selective estrogen receptor modulators (SERMs). A large number of compounds possessing TAE residue in an acyclic, cyclic or as a modified TAE have been investigated, leading to the development of drugs such as clomiphene for fertility induction, centchroman as a contraceptive, tamoxifen and the related derivative tormifene for breast cancer, raloxifene for osteoporosis and many more candidate drugs. This review discusses the potential of the triarylethylene pharmacophore in the development of drugs for estrogen-dependent conditions.

Introduction

The triarylethylene (TAE) pharmacophore present in the form of acyclic and cyclic molecular structures provides a class of compounds associated with various types of biological activities. Their effectiveness in areas such as fertility regulation, breast cancer, osteoporosis, CNS and cardiovascular disorders, lipid disorders, etc., is basically due to their action as estrogen agonists or antagonists. Some of the compounds elicit tissue-selective effects and are termed selective estrogen receptor modulators (SERMs). Such compounds show promise in tissue-oriented estrogen replacement therapy (ERT). However, only a few of these agents have entered the clinic so far. The present review highlights the potential of the TAE pharmacophore present in various molecular frameworks, with special reference to its use in fertility regulation, breast cancer and osteoporosis. Only compounds which exhibit promising biological activity are discussed here.

Historical developments

The introduction of the TAEs as a class of estrogens was an outcome of molecular modification of the diethylstilbestrol (DES) (**1**), identified as a potent estrogen by Dodds *et al.* (1, 2) in 1938. The replacement of an ethyl group in DES by an aryl in triarylethylene **2** produced compounds with some selectivity in estrogen action. Compared to the endogenous estrogen estradiol (**3**), TAEs possessed oral activity and more prolonged biological effects. A detailed study of structure-activity relation-



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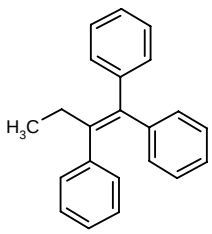

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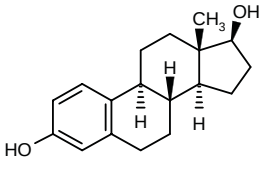


(**3**)

ships (SAR) of TAE derivatives can be found in a review by Grundy (3).

Development of molecules containing the triarylethylene pharmacophore

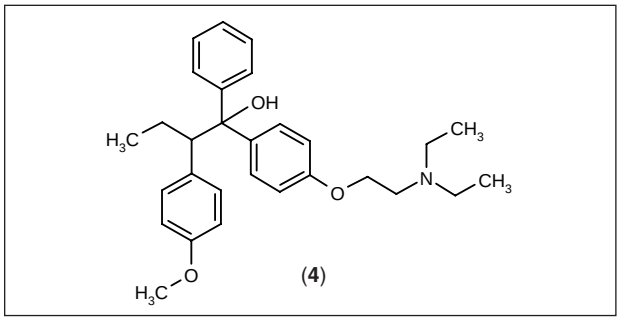
Acyclic molecules containing the triarylethylene pharmacophore

The TAE molecules did not find much use as estrogens. However, their estrogenic profile compared to estradiol or DES raised the possibility that molecules could be designed with a somewhat selective estrogenic response, referred to as atypical estrogens.

The most significant milestone in the development of TAEs was the finding that when an appropriate tertiary aminoalkoxy group was introduced at an appropriate position on a phenyl residue, the molecule presented an estrogen-antagonist effect. Initially, the use of such compounds was aimed at developing fertility-regulating agents. Later on, the interest tilted more towards the development of anticancer agents, as well as other estrogen-dependent areas, as discussed later. The first antiestrogenic TAE, MER-25 (**4**) (4), was found to antagonize the effects of estrogens when administered concurrently and possessed a weak estrogenic profile. It inhibited fertilization when given to female rats (5, 6). This observation opened up the possibility of the use of estrogen antagonists as fertility-regulating agents. However, in preliminary clinical trials, MER-25 showed poor efficacy and had toxic effects, leading to discontinuation of the trials (7).

A number of tertiary aminoalkoxy derivatives of triphenylethylenes, -ethanes and -ethanols synthesized subsequently during this period (8) showed antiestrogenic, antiinflammatory and cholesterol-lowering activities. Antifertility activity was also observed in MRL-37 (**5**) (9). A compound of particular interest was clomiphene (MRL-41, **6**) (10). It showed antigonadotropic, estrogenic and antiestrogenic properties in rats (11) and was effective in blocking the action of endogenous and exogenous steroidal and nonsteroidal estrogens in rats, mice, rabbits and monkeys (12). However, when administered in women, it was found to aid in the induction of ovulation. The *trans*-isomer of clomiphene, enclomiphene, is predominantly antiestrogenic, while the *cis*-isomer, zuclomiphene, is estrogenic. It was found to prevent bone mineral loss in ovariectomized rats (13, 14) without any adverse uterine or pituitary consequences. Long-term administration to ovariectomized rats prevented osteoporosis and maintained cortical bone thickness and total body calcium (15). The clinical use of clomiphene is discussed in a later section of this review. MER-25, MRL-37 and MRL-41 all elicited antifertility activity in rats (5, 6).

The nitroethylene derivative CN-55945-27 (**7**) (16) was found to be antizygotic. It prevented pregnancy in mice (50 mg/kg), rats (25 mg/kg) and dogs (250-500 mg/kg) when fed continuously in the diet. It showed weak uterotrophic, antiestrogenic and antideciduogenic activities. It stimulated primary luteinizing hormone (LH) secretion but had no effect on follicle-stimulating hormone (FSH) release. Its antiimplantation effect is possibly due to its antiestrogenic and/or luteolytic action via LH stimulation (17).



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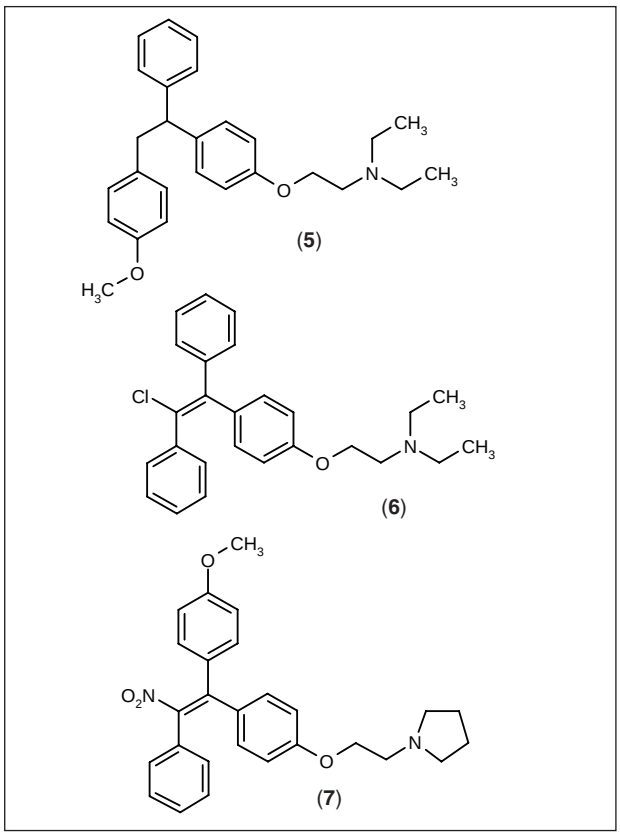

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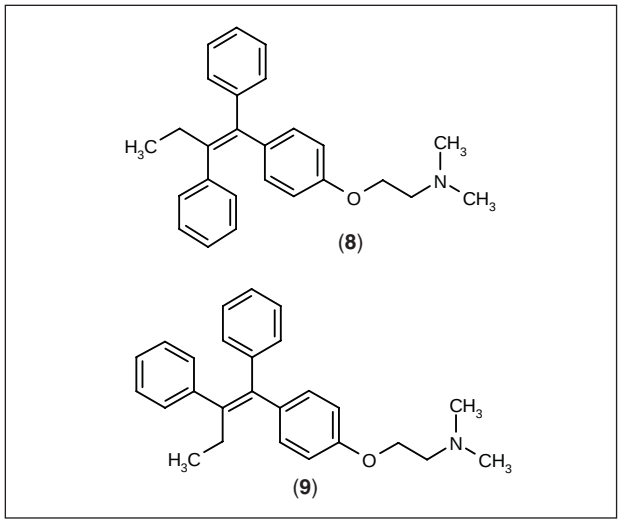


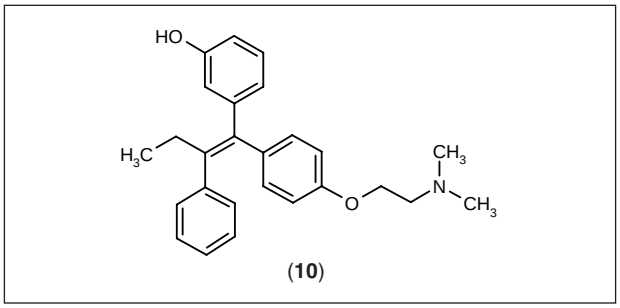
The most studied triarylethylene derivative is tamoxifen (**8**) (18-20), a *Z*-isomer with potent estrogen-antagonist, weak estrogenic and mildly antigonadotropic activity. However, its *E*-isomer (**9**) was a potent estrogen in rat and mouse models, antigonadotropic and prevented implantation when administered on days 1-4 of pregnancy at doses inducing vaginal cornification.

Tamoxifen caused bone resorption in birds and prevented further development of medullary bone in estrogen-treated capons, inhibiting osteoblastic function and inducing osteoclastic resorption (21). However, in young adult rats, tamoxifen acts as a potent inhibitor of bone resorption and prevents the increase in osteoclast number and resorbing surface area (22). In ovariectomized mice, it acts like a true estrogen, prevents bone loss and also any decrease in bone density or calcium and the phosphorus content of femur (23).

Tamoxifen is the first estrogen agonist/antagonist identified to inhibit bone loss in postmenopausal women with breast cancer (24, 25) and in the ovariectomized rat model (26, 27). In clinical trials in postmenopausal women with breast cancer, tamoxifen, alone or in combination with prednisolone, prevented bone loss, particularly in steroid-treated patients (28).

The mechanism of action of tamoxifen in bone metabolism may involve a direct interaction with estrogen receptors (ER) present in the bone cells (29-32). Tamoxifen has also been shown to lower cholesterol in ovariectomized and intact rats (33). The use of tamoxifen in breast cancer is discussed in a later section.

In order to improve upon the pharmacological profile of tamoxifen, its molecular modification was attempted, resulting in the development of newer TAE derivatives possessing different estrogen-agonist/antagonist profiles (34), including pure estrogen antagonists (35). Some of the derivatives of tamoxifen which have been identified for drug development are described below.



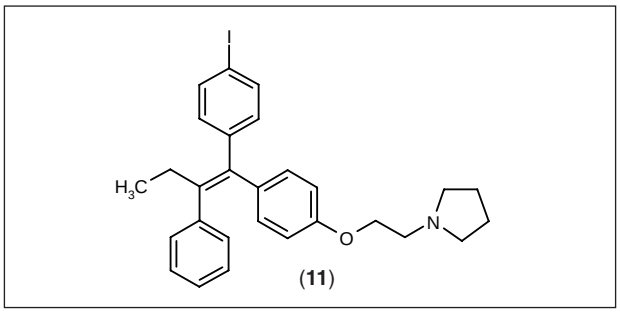
1. Droloxifene

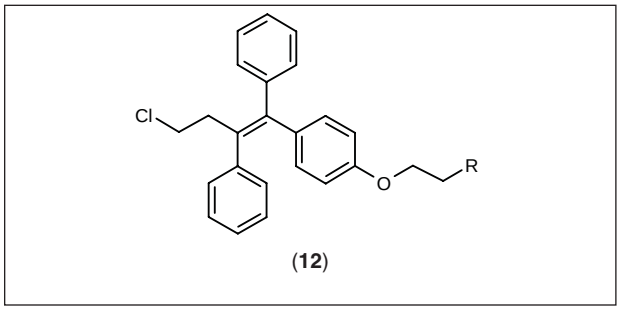
Droloxifene (**10**), a 3-hydroxyphenyl derivative of tamoxifen, was synthesized by Schickaneder *et al.* (36). It binds to ER with 10-60-fold higher affinity than tamoxifen and showed much lower intrinsic estrogenicity as compared to tamoxifen in rat uterus. Its estrogenicity is about 3.3% of that of estradiol. In *in vitro* studies, droloxifene showed a greater antiestrogenic effect on the proliferation of ER-positive breast cancer cells (MCF7 and ZR-75) (37-39). *In vivo* studies displayed increased growth-inhibitory activity of droloxifene in different tumors of animal (R 3230Ac and 13762) and human origin (T61). It was found to be less toxic than tamoxifen and did not show any carcinogenic or mutagenic effects in *in vivo* and *in vitro* studies. In a phase II trial in 268 postmenopausal patients with metastatic breast cancer, droloxifene at doses of 20, 40 or 100 mg/day produced response rates of 30%, 47% and 44%, respectively, with mild toxicity (40). Similar results were reported in other multicenter, double-blind, randomized trials in patients with advanced breast cancer (41, 42).

Droloxifene effectively prevents bone loss and lowers total serum cholesterol without causing uterine hypertrophy in both growing and aged ovariectomized rats (43), and it was reported to be effective in the treatment of endometriosis and uterine fibroid disease (44). However, clinical trials with droloxifene for breast cancer (phase III) and osteoporosis (phase II) were discontinued.

2. Idoxifene

Idoxifene (**11)** (45a, 45b) was essentially designed as an approach to preventing the metabolism of tamoxifen to





(**12**)

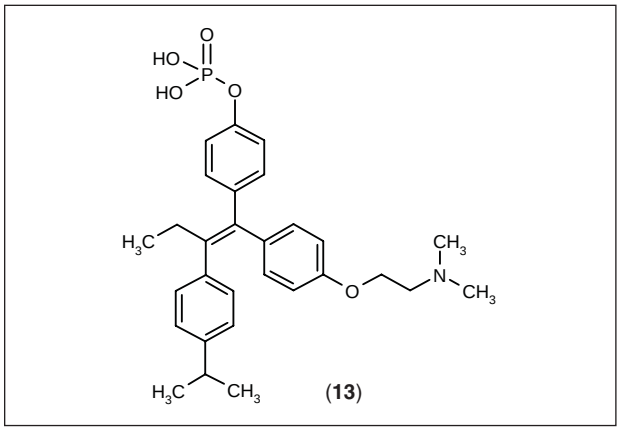
4-hydroxytamoxifen, which is rapidly inactivated through glucuronide formation. It also shows a reduced rate of side-chain *N*-oxidation and demethylation (46). Idoxifene, which had reached phase III clinical trials for the prevention of osteoporosis and was also under clinical evaluation for breast cancer (47), was discontinued for both treatment indications.

3. Toremifene

Toremifene (**12,** R = NMe_2) was synthesized by Farmos (48) and was designed to avoid the formation of α'-OH-tamoxifen, a metabolite responsible for the genotoxicity caused by tamoxifen. It has been introduced for the adjuvant therapy in earlier stages of breast cancer (see later section).

4. TAT-59 (miproxifene)

TAT-59 (miproxifene) (**13**) is a prodrug that requires dephosphorylation to the active metabolite of tamoxifen, 4-OH-tamoxifen, that was synthesized (49a, 49b) and developed for the treatment of breast cancer. It has been shown to inhibit the growth of ER-positive DMBA-induced rat mammary carcinomas (50a-50c) and it inhibited the growth of MCF7 cells in a concentration-dependent manner. Relative activity was on the order of TAT-59 > droloxifene > tamoxifen (39). The active dephosphorylated form binds with high affinity to ER (51).



(**13**)

5. Ospemifene (FC-1271a)

The deaminohydroxy metabolite of toremifene, ospemifene (FC-1271a) (**12,** R = OH), is a novel triphenylethylene compound with a tissue-selective profile, having estrogen-like effects on bone and the cardiovascular system and antiestrogen-like effects in uterus and breast, which makes it a good candidate drug for the prevention of osteoporosis. In postmenopausal women, it was effective in reducing climacteric symptoms. It specifically binds to ERα and ERβ subtypes with affinity similar to that of toremifene and tamoxifen (52, 53a, 53b).

Cyclized molecules containing the triarylethylene pharmacophore

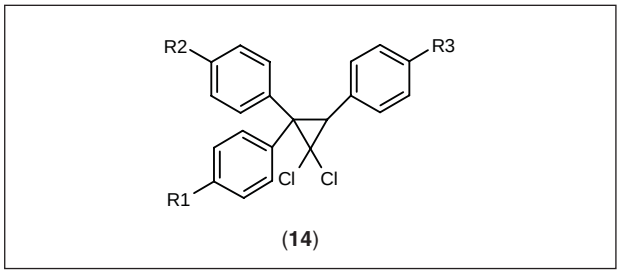
The TAE pharmacophore present in the form of carbocyclic or heterocyclic structures has also provided a number of compounds possessing estrogen-agonist/antagonist activity. Some significant compounds are discussed below.

1. Triarylcyclopropyl derivatives

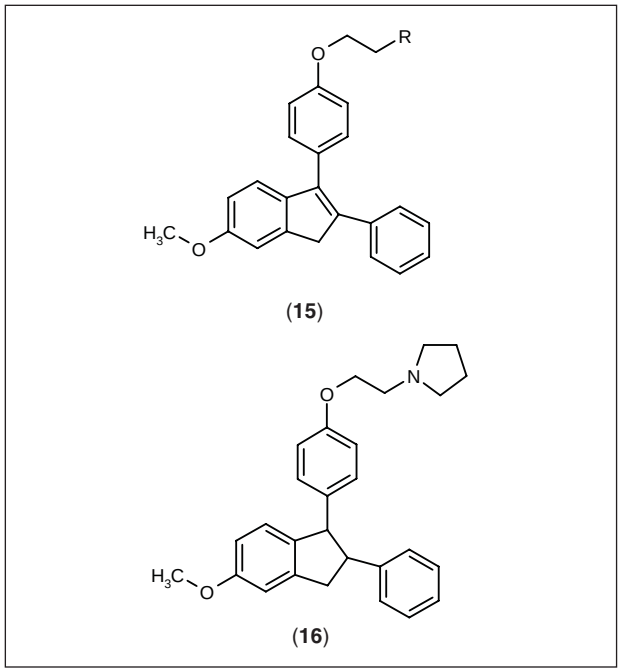
Antiestrogenic activity has been reported in the *gem*-dichlorotriarylcyclopropyl compounds **14**. Some of the compounds in this series inhibit the growth of ER-positive human breast cancer MCF7 cells in culture (54, 55). Interestingly, the usual ω-tertiary aminoalkoxy group was not essential for antiestrogenic activity in these compounds and a benzyl substituent could induce this effect.

2. Diarylindenes and -indanes

The 2,3-diarylindenes **15** were the first group of cyclized TAEs (56, 57) prepared as estrogen antagonists. It was observed that the presence of a nitrogen-containing basic side-chain on the 3-phenyl residue in this group of compounds was essential for antifertility activity and that the preference for the basic residue was on the order pyrrolidino > diethylamino > morpholino attached through an ethoxy chain. In this series, U-11555A (**15**, R = NEt_2) was investigated extensively in rodents (58, 59). It possessed weak uterotrophic, antideciduogenic and antiestrogenic activities, and was devoid of progestational and



(**14**)



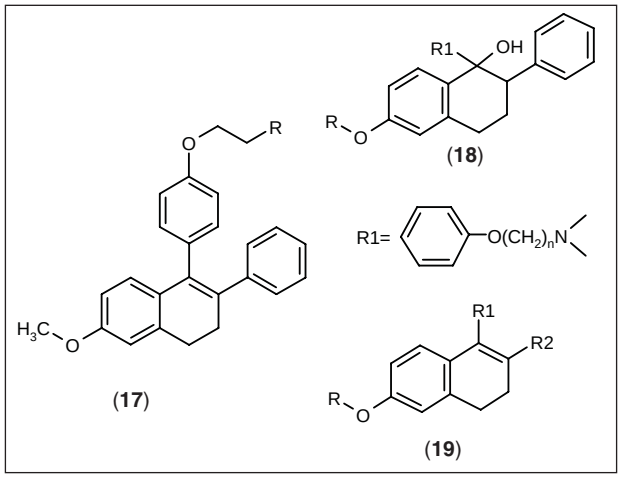
antigonadotropic effects. U-11555A prevented implantation in rabbits and rats at doses of 15 and 0.1 mg/kg, respectively (57). However, the corresponding pyrrolidino compound **15** (R = NC_4H_8) was most active in the series, with activity at 0.025 mg/kg in rats.

In the indane series, 1,2-*cis*- and 1,2-*trans*-**16** showed antiimplantation activity at 0.5 and 0.25 mg/kg, respectively. These compounds were 152 and 108 times less estrogenic, respectively, compared to ethinylestradiol and showed antiestrogenic properties (60, 61).

3. Diarylnaphthalenes

Potent antifertility activity was observed in 1,2-diphenyl-3,4-dihydronaphthalene compounds possessing a TAE chromophore (62, 63). Two compounds in this series, U-11100A (**17**), (R = NC_4H_8), nafoxidine) and U-10520A (**17**, R = NEt_2), were investigated for antifertility activity (64). The compounds were 100% effective in preventing pregnancy in rats at respective doses of 0.025 and 0.25 mg/kg given orally on days 1-5. The compounds possessed mild uterotrophic, antiestrogenic and antideciduogenic activities and were devoid of antigonadrotropic activity. In rhesus monkeys, oral administration of nafoxidine caused only partial inhibition of pregnancy even at a high dose of 250 mg/day. No fetal abnormalities were observed (59). In rabbits, it did not exert a blastotoxic effect (59).

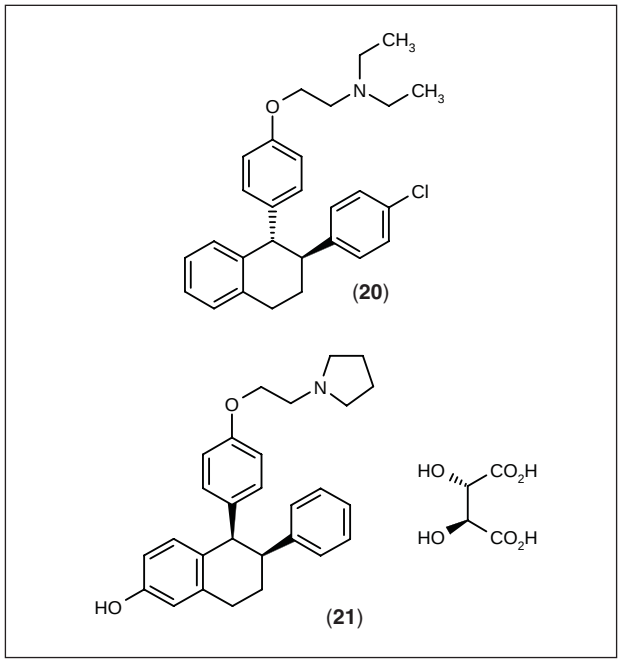
Basic ether derivatives of 1,2,3,4-tetrahydro-1,2-diaryl-1-naphthols **18** (65), obtained as intermediates during the preparation of 1,2-diaryl-3,4-dihydronaphthalenes, also elicited antifertility activity. Substitution of the 1-phenyl bearing the basic side-chain with a pyridyl

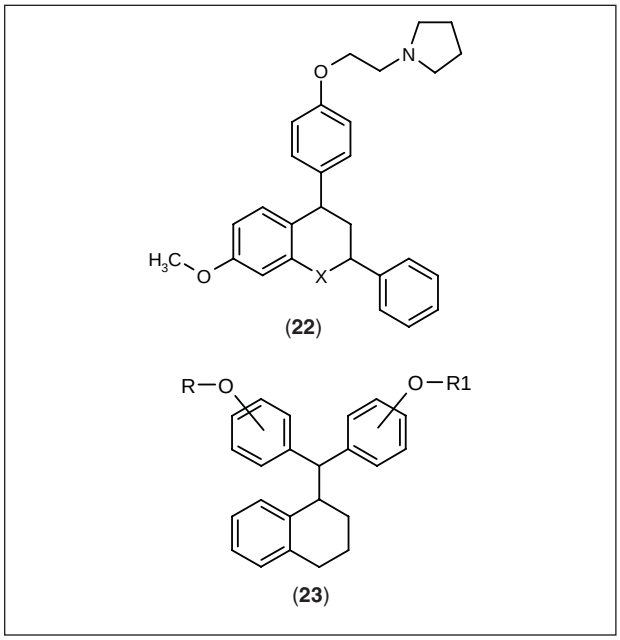


(**19**, $R_1 = C_5H_5N$, R_2 = Ph) retained activity. However, substitution of the 2-phenyl group by pyridyl (**19**, R_1 = Ar, $R_2 = C_5H_5N$) diminished activity.

Of the *cis*- and *trans*-isomers of 1,2-diaryl-1,2,3,4-tetrahydronaphthalenes (62), the *trans*-isomer **20** was found to be most active and prevented implantation in rats at an oral dose of 20 mg/kg/day. CP-336156 (**21**), a novel, third-generation, orally active, nonsteroidal ER agonist/antagonist was developed by Pfizer (66a, 66b) for the prevention and treatment of osteoporosis (67) in postmenopausal women. It displays high and selective binding affinity for the human ERα (68a, 68b).

Interestingly, shifting the phenyl moiety of 1,2-diaryltetrahydronaphthalene to position 3 (**22**, X = CH_2) abrogated the antifertility activity (69). Similarly, 2,4-diarylchromans (**22,** X = O) (70) were also found to be inactive, whereas corresponding 3,4-diarylchromans, discussed





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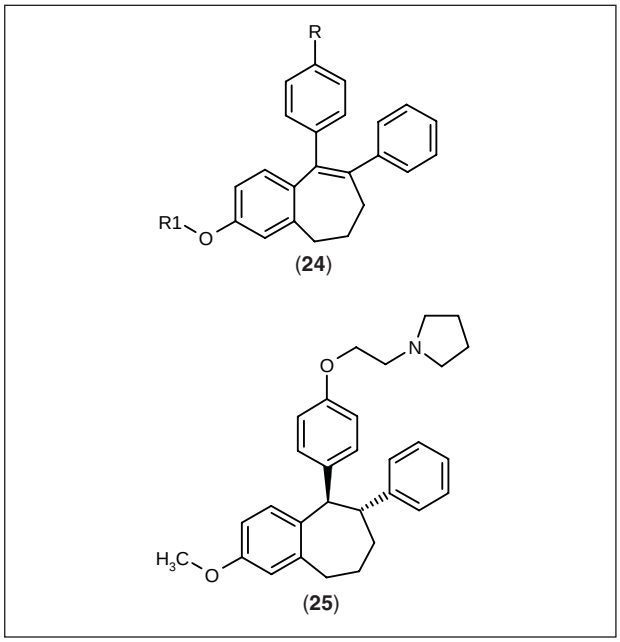


(**24**)

(**25**)

later, showed significant activity. This and other studies reported by Lednicer *et al.* with the 2-pyridyl compound reported above (65), would suggest the importance of the TAE pharmacophore and the need for a basic residue at an appropriate place in space.

The TAE pharmacophore, present in the form of diaryltetrahydronaphthylmethane (**23**, R_1 = basic chain) provided compounds with significant antifertility activity (70, 71). Bisphenol derivatives of similar structure (**23**, R = R_1 = H) have been reported to bind to estrogen receptors with high affinity (72).

4. 1,2-Diarylbenzosuberan derivatives

In the carbocylic system, antifertility activity was retained when TAE formed part of a 7-membered ring. Thus, the 1,2-diphenylbenzocycloheptene derivatives **24** (73) were potent estrogens in rats. The basic ether derivative of the reduced compound **25** was found to inhibit implantation at 0.2 mg/kg on days 1-5 and 1.5 mg/kg as a single oral dose on day 1 in rats (74).

5. Heterocyclic triarylethylene chromophores

The TAE chromophore as a part of a heterocyclic ring has been studied extensively. Significant antifertility activity has been reported in the 2,3-diarylindole derivatives **26** (75, 76).

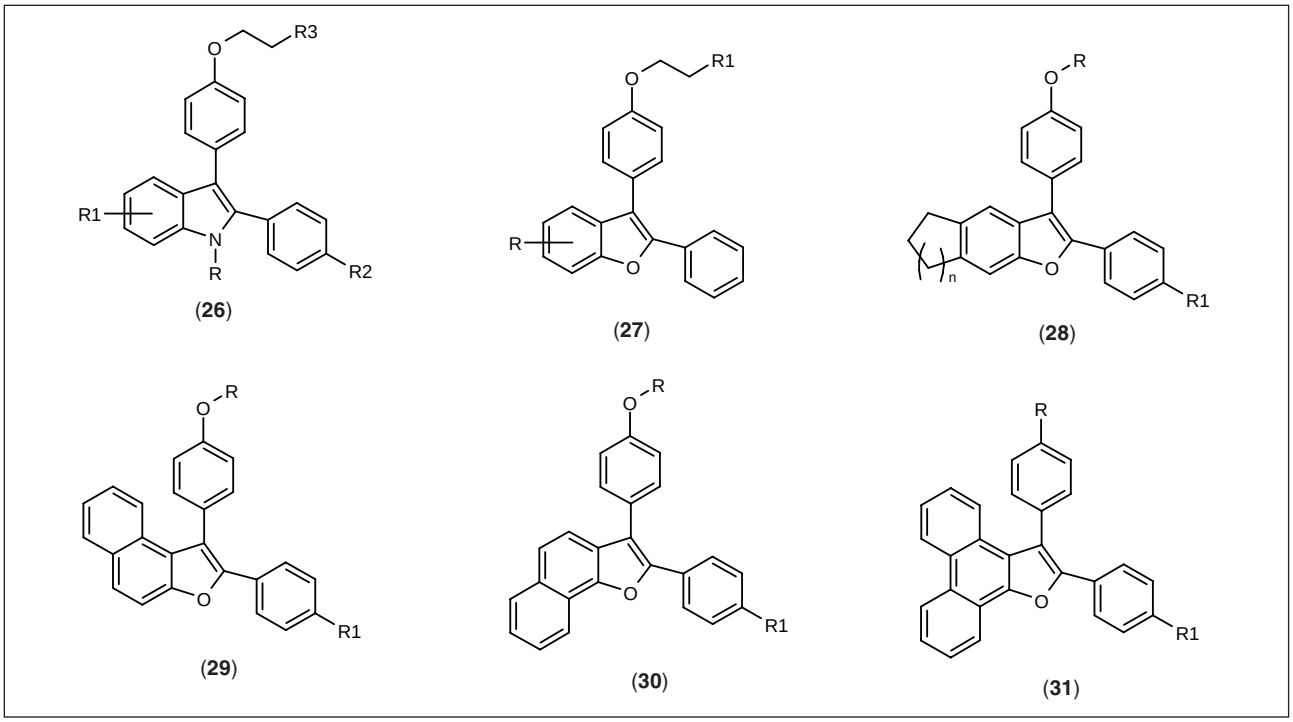
Two of the basic ether derivatives (**26**, R = R_1 = R_2 = H, R_3 = NEt_2/NC_4H_8) inhibited implantation at 5 mg/kg in rats.

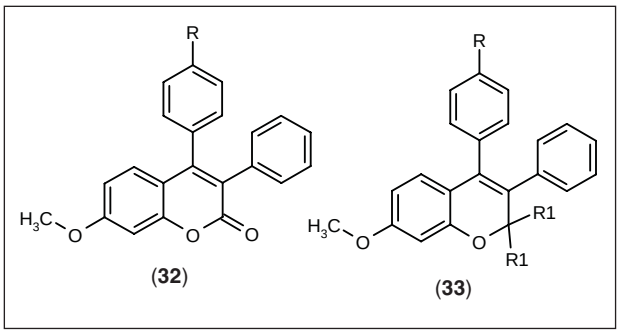
In the series of basic ethers of 1,2-diphenylbenzofurans, the most active compound (**27**, R = 6-OMe) R_1 = NC_4H_8 was active as an antiimplantation agent at a dose of 4 mg/kg in rats (77).

A structure-activity relationship (SAR) study with various benzofurans, 5,6-polymethylene-substituted benzofurans (**28**), naphtho[2,1-*b*]furans (**29**), naphtho[1,2-*b*]-furans (**30**) and 2,3-diarylphenanthrofurans (**31**) suggested that the antifertility activity of the compounds arising from the TAE pharmacophore also depends upon the overall structural geometry of the molecule. Thus, while derivative **28** (R = $(CH_2)_2NC_4H_8$, R_1 = H, n = 2) was active at 2 mg/kg in preventing implantation in rats, the naphtho-[2,1-*b*]furan **29** (R = $(CH_2)_2NC_4H_8$, R_1 = H) and naphtho[1,2-*b*]furan **30** (R = $(CH_2)_2NC_4H_8$, R_1= H) were active at doses of 2 and 20 mg/kg, respectively (78). Phenanthrofuran derivatives **31** did not show any significant activity (79).

The benzofuran **27** (R = 6-OMe, R_1 = NC_4H_8) and the naphthofuran **29** (R = $(CH_2)_2NC_4H_8$, R_1 = H) were investigated for development as oral contraceptives. Both compounds showed weak estrogenic and antiestrogenic properties and were devoid of other hormonal properties, with a virtually inert endocrine and gross pharmacological profile (78). In rats, the compounds caused fetal resorption without producing genital abnormalities or teratogenicity. Postnatal sexual development and subsequent fertility remained normal. However, these compounds were not pursued further as they produced liver toxicity.

In the benzopyran series, basic ethers of the 3,4-diarylcoumarins **32** (80-83), -chromenes **33**, -*cis*- and -*trans*-chromans **34** and **35** (84-88) elicited antifertility and antiinflammatory activity. The antifertility activity was found to be of low order in coumarins, whereas chromenes and chromans showed significant activity (88). The bulk and orientation of substituents played a major role in determining biological activity. Whereas in chromenes an alkyl substituent at C-2 in increasing order





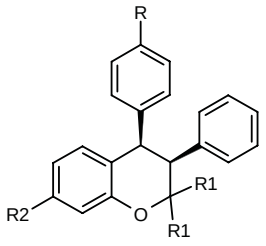
of bulk had a detrimental effect, in chromans optimum activity was observed in *trans*-2,2-dimethylchromans. Furthermore, the axial methyl at C-2 (α-orientated) in (3*R*, 4*R*)-*trans*-3,4-diarylchromans (**35,** R = $O(CH_2)_2NC_4H_8$, R_1 = Me) conferred a 8-fold higher relative binding affinity (RBA) for the ER as compared to the corresponding (3*S*,4*S*)-enantiomer **35** (89), in which the axial methyl is β-oriented. Replacement of the ω-tertiary aminoethoxy substituent present at the *para*-position of the 4-phenyl residue by a 3-(substituted amino)-2-hydroxypropyloxy group caused significant enhancement of antifertility activity (83). All of these compounds were found to be partial estrogen antagonists.

The *trans*-chroman **35** (R = $O(CH_2)_2NC_4H_8$, R_1= Me) showing potent antifertility activity in rodents had a favorable hormonal profile. Known as centchroman, this compound was studied in detail. Centchroman is a partial estrogen antagonist and a true SERM. Acting as an antiestrogen at the uterine tissue level, it inhibits implantation and was introduced as the first oral nonsteroidal contraceptive (see later section). Centchroman has also been found to be very effective in the treatment of dysfunctional uterine bleeding (DUB) (90) and was introduced for the treatment of DUB under the name Sevista. In 60 patients with abnormal uterine bleeding (AUB), treatment with centchroman was not associated with endometrial hyperplasia or ovarian enlargement (91).

Centchroman also exerts an estrogen-agonist effect in bone tissue and prevents bone loss by directly inhibiting the bone-resorbing activity of osteoclasts (92a, 92b). The *l*-enantiomer of centchroman (levormeloxifene) was found to inhibit bone loss and arterial cholesterol accumulation in estrogen-deficient animal models, without stimulating endometrial glands or epithelium (93-96). In a double-blind study in postmenopausal women (45-65 years of age), levormeloxifene administration produced estrogen-like bone-preserving effects (97). In phase II/III clinical trials in approximately 4,000 elderly women exposed to levormeloxifene, despite the desired efficacy on bone and lipids, a somewhat higher incidence of urinary incontinence and utero-vaginal collapse was observed. Considering the overall risk profile of the compound, the trials were discontinued (98).

Another compound from this series, **34** (R = $O(CH_2)_2NC_4H_8$, R_1= R_2 = H), reported earlier as a racemate (87), was recently resolved. The (–)-isomer NNC-45-0781 was found to be effective in the treatment and prevention of osteoporosis. The product is under active development in phase II/III clinical trials (99a, 99b).

Corresponding naphthocoumarins **36** and naphthochromenes **37** (100) did not possess any significant antifertility activity.



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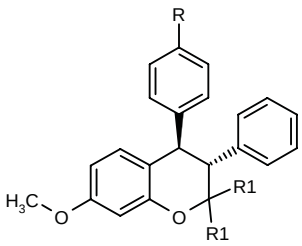

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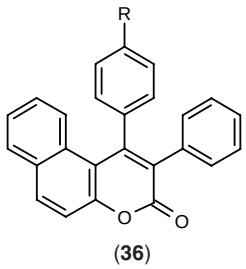

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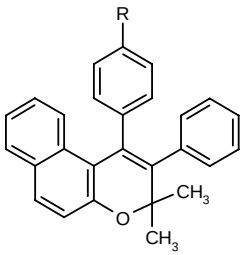

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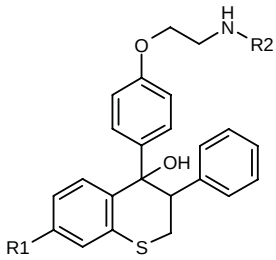

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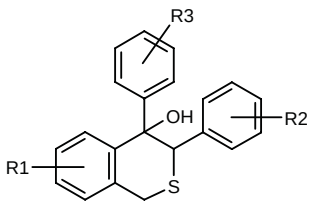


(**39**)

Triarylethylene residues in the form of thiochromans **38** (101) and isothiochromans **39** (102) were prepared as potential antiestrogens. In the latter series, compound **39** (R_1 = 7-OMe, R_2 = H, R_3 = $O(CH_2)_2NC_4H_8$) inhibited pregnancy in mice when administered orally at 0.1 mg/kg.

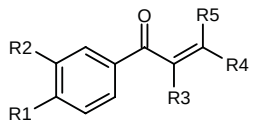
Modified triarylethylenes

The TAE molecule is an extension of DES, which closely resembles estradiol and therefore fits into the ER in a manner similar to estradiol to elicit estrogenic effects. The third aryl residue, usually carrying a basic chain, is responsible for the estrogen-antagonist activity (see later section).

Exploitation of the TAE nucleus extending it to molecular structures showing partial deviation from the TAE chromophore in both acyclic as well as cyclic forms, has led to the synthesis of a large number of compounds with potent estrogen-antagonist and tissue-selective estrogenic actions.

Acyclic variants

In an attempt to enhance the antiestrogenic potential of TAEs, a number of structural modifications have been attempted. The acrylophenone derivatives 1,2,3-triaryl-2-propen-1-ones (TAPs), prepared by this institute (103), showed promising activity. Of these, **40** (R_1 = 4-(2-pyrrolidinoethoxy)phenyl, R_2 = H, R_3 = R_4 = Ph, R_5 = 4-Cl-Ph) inhibited implantation in rats at a dose of 1 mg/kg. *Erythro*-isomers of 1,2,3-triarylpropan-1-ones **41** (104) substituted with a basic chain at the *para*-position of the



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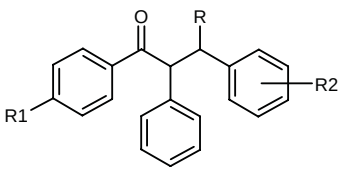

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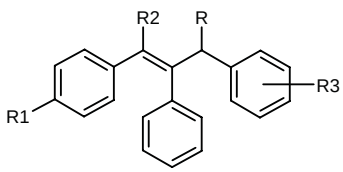

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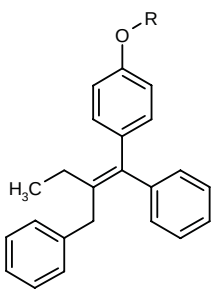

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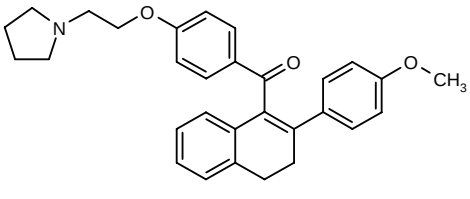

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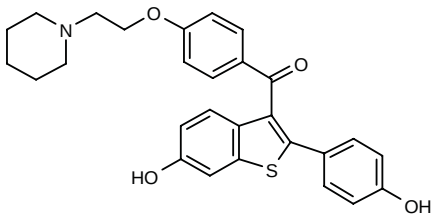
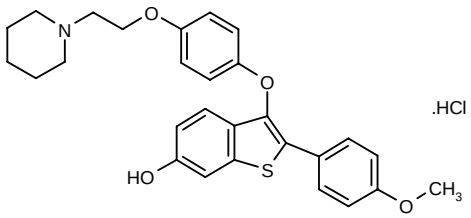


(**45**)



(**46**)

1-phenyl residue and an alkyl substituent at C-3 were found to be more potent in inhibiting fertility as compared to the corresponding *threo*-isomers and possessed significant antiestrogenic activity (105).

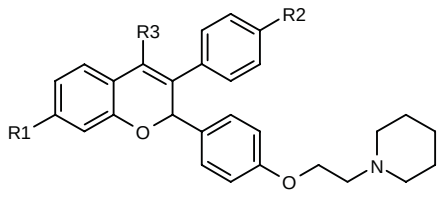
Significant antiimplantation activity was also found in 1,2,3-triarylpropenes **42** (106), of which **42** (R = $O(CH_2)NC_4H_8$, $R_1 = R_2 = H$, R_3 = 3-OMe) was active at 0.5 mg/kg in rats. Comparable or lower IC_{50} values for antiproliferative effects against the human breast cancer MCF7 cell line have been obtained with compounds of structure **43** wherein R is a basic side-chain (107).

Cyclic variants

The TAE nucleus in the cyclized form has provided potent antiestrogens. Thus, trioxifene (**44**) (108) significantly inhibited the uterotrophic response to estrone in mice at 1 mg/day, a dose associated with weak uterotrophic activity. It prevented fertility in rats at 5 mg/day.

The benzothiophene derivative raloxifene (**45**) (109), developed by Lilly, was found to be a potent antiestrogen and to selectively inhibit bone loss. It is less effective than tamoxifen as an antitumor agent (110, 111) and has been introduced for the treatment of osteoporosis. Its clinical use is discussed in a later section.

Replacement of the carbonyl group of raloxifene by oxygen resulted in the formation of arzoxifene hydrochloride (**46**), also synthesized by Lilly (112a, 112b, 113). This



(**47**) $R_1 = R_2 = R_3$ = H CDRI 85/287
(**48**) $R_1 = R_2$ = OH; R_3 = Me EM 343 (*dl*)
(**49**) $R_1 = R_2$ = OH, R_3 = Me (EM 652, *S*-enantiomer)
(**50**) $R_1 = R_2$ = OPivaloyl, $R_3 = CH_3$ (EM 800, S enantiomer)

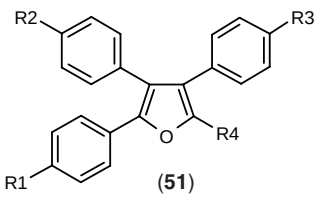
product has shown promise as a SERM for the treatment of postmenopausal syndrome and cancer (113). Arzoxifene is in phase II clinical testing for the treatment of ovarian and endometrial cancers (114), and has entered phase III trials for breast cancer.

In another modification, triarylpropanes and triarylpropenes in cyclized form provided the potent estrogen antagonists 2,3-diarylchromenes **47-50**. The first compound in this series, **47** (CDRI 85/287), reported by this institute (115-117), was found to effect complete inhibition of estradiol-stimulated uterine growth. It was also effective in preventing bone loss in a rat model (118).

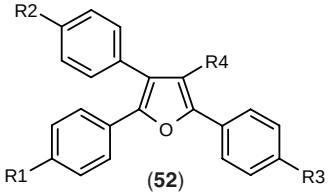
The *R*-enantiomer of **47** (119) was found to potently inhibit implantation. The 4-methyl derivative EM-343 (**48**) (120a) and its (*S*)-enantiomer EM-652 (**49**) (120b) were also highly antiestrogenic. EM-800 (**50**)**,** the pivaloyl ether prodrug of EM-652, was tested for the prevention of bone loss (121). It acts as a pure antagonist and blocks the transcriptional functions AF-1 and AF-2 of ERα and ERβ (122).

Antifertility activity was observed in the 2,3,4-triarylfurans **51**. Both phenolic and basic chain-substituted derivatives prevented implantation ($R_1 = R_2 = R_4 = H$, R_3 = OH: 2 mg/kg; $R_1 = R_3 = R_4 = H$, $R_2 = O(CH_2)_2NC_4H_8$: 1 mg/kg; $R_1 = O(CH_2)_2NC_4H_8$, $R_2 = R_3 = R_4 = H$: 5 mg/kg) (123a, 123b).

3-Alkyl-2,4,5-triarylfurans **52** with a substituted basic side-chain were prepared and evaluated (124) for ER binding. High affinity for the ER was observed for



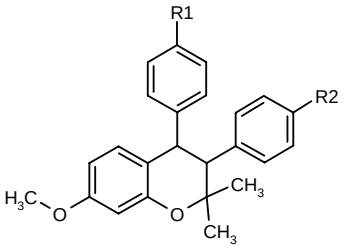
(**51**)



(**52**)

compounds with a basic side-chain on the C-4 phenol, which were found to be ERα-selective antagonists.

A study of the active and inactive structural variants discussed above would indicate that the primary criterion that the structure has to meet to show agonist or antagonist activity is its capability to adjust into the groove where DES fits. In addition, a basic side-chain on a phenol at an appropriate place, as discussed below, is requireded for antagonist activity.



(**53**)

Mechanism of action of triarylethylenes

The mechanism of estrogenic activity has been discerned to a large extent (125-129). A primary determinant of whether a ligand is an estrogen agonist or antagonist is its binding to the ER. The present discussion will be limited to the interaction of TAE with the ER for inducing estrogen-agonist or -antagonist effects.

As mentioned above, the TAE nucleus is derived from the DES structure, with an additional phenyl residue attached to it. Diethylstilbestrol elicits a frank estrogenic response due to its structural resemblance to the endogenous estrogen estradiol. It has been shown in X-ray studies with ligand-bound receptor (129) that both these molecules bind to the ER through a hydrogen bond with His-524 on one end and three units –Glu-353, Arg-394 and a water molecule– on the other end. This binding causes repositioning of helix 12 at the C-terminal end of the ligand-binding domain (LBD) to interact with helices 3, 5/6, so as to enclose the ligand in the LBD groove, necessary for AF-2 activity for initiating an estrogenic effect. In estrogen antagonists, the presence of an aminoethoxy chain at an appropriate position, as in tamoxifen, nafoxidine, centchroman, raloxifene, *etc.*, is of crucial importance. The amino residue present on raloxifene (**44**) binds to Asp-351 of the ER. This prevents helix 12 from closing onto helix 3, thereby positioning the ligand in the LBD groove for AF-2 activity. It has been demonstrated (128) that 4-hydroxytamoxifen also behaves in a similar manner but binds to Asp-351 via a weaker interaction. Removal of the aminoethoxy chain abolishes the estrogen-antagonist effect.

The molecular geometry of the ligand and the manner in which it binds to the LBD determine the biological activity of the molecule. An interesting example is that of (*Z*)- and (*E*)-tamoxifen (**8** and **9**); the former is an antagonist while the latter is an agonist. It could be seen from their structures that in the *Z*-isomer, the primary interaction with the ER involves the *trans*-stilbene unit comprised of unsubstituted phenyl rings, leaving the basic chain-substituted phenyl to interact for antiestrogenic effect. However, in the *E*-isomer, the *trans*-stilbene core for primary binding is generated only through deblocking of the chain, needed for antiestrogenic activity. Similarly, in *trans*-3,4-diarylchromans **53,** the antiestrogenic property of the compound is lost when the basic chain is shifted from 4-phenyl (**53,** R_1 = pyrrolidinoethoxy, R_2 = H) to 3-phenyl (**53**, R_1 = H, R_2 = pyrrolidinoethoxy).

Drugs emerging from the triarylethylene pharmacophore or its modified variants

There are certain drug molecules on the market today, and many more in the pipeline at various stages of drug development, that possess the TAE pharmacophore or modified variants. An update on the marketed drugs clomiphene, centchroman, tamoxifen, toremifene and raloxifene is presented below.

Clomiphene: a drug for fertility induction

Clomiphene (chloramiphene, Clomid, MRL-41, **6**) was introduced in 1968 as the first TAE to enter the drug market. It is still the first choice for ovulation induction. Its *trans*-isomer, enclomiphene, is a partial estrogen antagonist and the *cis*-form, zuclomiphene, is an agonist (130). In rodents, clomiphene exhibited estrogen-agonist and -antagonist effects (10, 11, 131, 132). It inhibited fertilization in mice and rats by its gonadotropin-suppressing, ovulation-inhibiting and blastotoxic effects (10). At lower doses, clomiphene stimulated the secretion of gonadotropins, particularly LH (133). It was found to stimulate ovulation in pseudopregnant rats when given at low doses (134, 135).

In preclinical studies, clomiphene proved to be safe. Metabolism studies carried out in immature rats (136) showed that most of the drug was eliminated as unchanged compound. 4-Hydroxyclomiphene, formed as a minor product, was the only detectable metabolite. No general teratogenic effects of clomiphene were observed in monkeys (137).

Clinical studies with clomiphene in women were first initiated for its fertility-inhibiting property observed in rodents. However, it was found (138a, 138b) that, on the contrary, it induced ovulation in anovulatory women and had no suppressive effects on ovulation in normal women. This led to the development of clomiphene citrate as a drug for ovulation induction. In clinical use, it is administered orally as a mixture of 38% zuclomiphene and 62% enclomiphene.

The mechanism of ovulation induction by clomiphene citrate as understood today is that it binds to the ER but does not induce the synthesis of new receptors, a process essential for the continuous binding of estradiol

to target cells, as well as the expression of estrogen action. As a result, a state of hypoestrogenicity created in the hypothalamus causes enhanced gonadotropin-releasing hormone (GnRH) release, followed by an increased secretion of gonadotropins, which facilitates ovulation.

In *in vitro* fertilization studies, clomiphene has been found to increase LH concentrations significantly in follicular and luteal phases (139a). It causes transient inhibition of aromatase activity, leading to stimulation of ovarian folliculogenesis, as seen with the aromatase inhibitor tetrozole (139b). Clomiphene citrate also stimulates estradiol synthesis in the ovaries, which in turn stimulates the formation of FSH and LH receptors in the granulosa cells of other small follicles, thereby promoting the development of additional follicles for ovulation. Clomiphene citrate causes induction of ovulation in ~70% cases and pregnancy in ~25%. This lower pregnancy rate could be due to: 1) changes in cervical mucus, making it hostile to sperm penetration; 2) follicular luteinization; or 3) an inadequate luteal phase.

The most common side effects of clomiphene use are ovarian enlargement and hot flashes and less frequent are breast tenderness, headache, nervousness, dizziness, nausea and vomiting, fatigue and temporary visual disturbances.

Centchroman: an oral contraceptive

Centchroman (ormeloxifene, **35,** R = pyrrolidinoethoxy, $R_1 = CH_3$) was the first nonsteroidal oral contraceptive developed by the Central Drug Research Institute in India. (81, 85, 141). The initial route of synthesis (85) was later modified (142).

Centchroman is a racemate and crystallographic studies (89) showed a molecular resemblance to the antiestrogens tamoxifen and nafoxidine. It has been resolved into its *d*- and *l*-enantiomers. *l*-Centchroman showed a 7-fold higher RBA for the ER and a corresponding difference in stimulation of uterine growth and antiimplantaion activity (143). *l*-Centchroman was found to have (3*R*,4*R*) absolute stereochemistry, which corresponds to that of *d*-estradiol (8β,9α) (144).

Centchroman prevented pregnancy in rats, mice, dogs and rhesus monkeys when administered as a single dose within 24 h of coitus. The antifertility effect was readily reversible (145). Centchroman did not interfere with endocrine function and had no gross pharmacological effects except for anorexigenic activity and antiinflammatory activity (equivalent to phenylbutazone) at about 40- and 64-fold the contraceptive dose (146, 147). In a 1-year study in rhesus monkeys, administration of the compound at 2 and 5 times the human contraceptive dose did not affect the hypothalamus-pituitary-gonadal axis (148). Weekly doses up to 120 mg given to women did not suppress ovulation and the hormone cycle (149).

When administered to rats postcoitally, centchroman slightly accelerated ovum transport, stimulated blastocyst formation and delayed zona pellucida shedding (150), but did not affect the viability of embryos (145, 150-152). These findings suggest that the contraceptive action of centchroman is primarily due to the induction of asynchrony between ovum transport and endometrium development for nidation.

In an *in vitro* metabolic study using rat liver homogenates, 8 metabolites were identified (153). Of these, 7-desmethylcentchroman, with 112% RBA to the ER and a similar order of antiimplantation activity as compared to centchroman, appears to be the active metabolite of centchroman.

The retention time of [^{14}C]-centchroman (154) administered to albino rats by the oral or i.v. route was found to be 120 h (155). In women, the terminal disposition half-life of the drug was about 170 h (156).

Centchroman showed no evidence of toxicity in animal studies (157) and was apparently devoid of mutagenic and carcinogenic effects. In recent studies (158-161), it has been shown to possess anticlastogenic and antimutagenic effects.

In clinical contraceptive use, centchroman 30 mg is given orally twice a week (beginning on day 1 of menses) for the first 12 weeks, followed by once-weekly doses. No side effects, except for prolongation of menstrual cycles to more than 45 days in 8% of cases, have been reported. In lactating women, centchroman was found to pass into the breast milk. However, the amount to which infants are exposed is only 2.5% of the maternal dose, which is unlikely to be of any physiological consequence to suckling babies (162).

Tamoxifen: a drug for breast cancer

Tamoxifen (**8**), was synthesized (163) and developed (18) by the former ICI (now AstraZeneca)**.** It has been in use for over 2 decades as the monocitrate salt for the endocrine therapy of breast cancer.

Tamoxifen competes with estradiol for binding to the ER (127) with an RBA of 2.0 (164). The metabolism of tamoxifen has been studied in mice, rats, dogs and rhesus monkeys (165-171). Unlike clomiphene, a large number of metabolites have been identified. Tamoxifene was found to be metabolized by liver microsomes of animals and humans to various products, mainly 4-hydroxytamoxifen, tamoxifen *N*-oxide and *N*-desmethyltamoxifen (172-176). Other metabolites obtained in minor amounts were 3,4-dihydroxytamoxifen, α-hydroxytamoxifen, α-hydroxytamoxifen *N*-oxide, α-hydroxy-*N*-desmethyltamoxifen, 4-hydroxytamoxifen *N*-oxide and tamoxifen epoxide (173, 177-180).

4-Hydroxytamoxifen is an active metabolite that binds to the ER with an affinity similar to that of estradiol. Most of the other metabolites, *i.e.*, 3,4-dihydroxytamoxifen, *N*-desmethyltamoxifen, α-hydroxytamoxifen and tamoxifen epoxide, bind covalently to proteins and DNA and are possibly responsible for its carcinogenic and

mutagenic effects and the development of resistance (181) to tamoxifen through receptor mutation.

In clinical use, tamoxifen is the drug of choice for the endocrine therapy of advanced breast cancer. The clinical efficacy of tamoxifen in breast cancer was first demonstrated by Cole *et al.* in 1971 (182). In a recent study, the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial recruited more than 13,000 women at high risk of developing breast cancer. The results showed that tamoxifen reduced the incidence of breast cancer in this high-risk population by 49% (183). The drug was well tolerated, and the dropout rate due to side effects was low. However, an increased rate of endometrial cancer was observed (184). There was a beneficial effect on bone but thromboembolic events increased in women over 50 years of age. As a result of this study, tamoxifen was approved in the United States for the prevention of breast cancer in women with an increased risk of developing breast cancer.

Toremifene

Toremifene (**12**, R = NMe_2) a derivative of tamoxifen, was designed to eliminate unwanted effects of tamoxifen arising from its metabolism. Toremifene is a potent antiestrogen. It binds to the ER and may exert estrogenic or antiestrogenic effects, or both, depending upon the experimental conditions.

Toremifene has *in vitro* antitumor activity in ER-positive MCF7 and ZR-75.1 cell lines. Toremifene is extensively metabolized, mainly in the liver. The main metabolite in serum is *N*-desmethyltoremifene.

Toremifene prevents prostate cancer in a transgenic mouse model of prostate adenocarcinoma (185). Acute and chronic toxicity studies have shown that most of the observed findings were related to its hormonal effects.
Toremifene is indicated for the treatment of metastatic breast cancer (186-189) in postmenopausal women with ER-positive or unknown tumors. It is similar to tamoxifen in clinical efficacy (190a, 190b) and has been marketed since 1988 in Finland as oral tablets (60 mg). Hypercalcemia and tumor flare have been reported in the first week of treatment with toremifene. It also causes endometrial cancer, as observed with tamoxifen. This drug may cause fetal harm when administered to pregnant women. The available data on toremifene suggest that the benefit/risk ratio of is comparable to that of tamoxifen in postmenopausal women with metastatic breast cancer.

Raloxifene: a drug for osteoporosis

Raloxifene (LY-139481, **45**) (191) is a new SERM that produces estrogen-agonist effects on bone, lipid metabolism and blood clotting, and estrogen-antagonist effects on uterine endometrium and breast tissue. It received approval from the U.S. FDA in 1997 for use in osteoporosis.

Raloxifene binds to the ER and activates it, while exhibiting tissue-specific effects distinct from estradiol (34). It has been found that raloxifene lowers total LDL cholesterol, but has no effect on HDL cholesterol levels (192). It does not cause endometrial hyperplasia or cancer. Moreover, raloxifene has been shown to possess beneficial effects on the cardiovascular system (193, 194).

Raloxifene did not alleviate early menopausal symptoms, such as hot flushes and urogenital atrophy, and may even exacerbate some of them (195). Raloxifene treatment in mice causes a dose-dependent increase in LH levels and associated changes in ovarian follicular morphology. This effect is, however, reversible on discontinuation of treatment (196).

The mechanism of action of raloxifene is not fully known. Its tissue-selective effects may involve differential interactions with the ER and cellular coactivators/repressors or at the DNA level (197). Raloxifene may also influence gene transcription, via the ER, by interacting with a DNA site other than the estrogen response element (ERE), known as the raloxifene response element (RRE) (198). *In vitro,* raloxifene binds to both ERα and ERβ with binding affinity of 46% at the human ERα and 26% at the rat ERβ (199), and stimulates transforming-growth factor TGF-β_3 (200).

Clinically, raloxifene is taken orally at a dose of 60 mg/day for the treatment and prevention of postmenopausal osteoporosis. Raloxifene is contraindicated in pregnant or lactating women, in patients with known hypersensitivity to raloxifene and in those with a history of thromboembolic events (201). The drug is well tolerated. Most of the adverse effects are mild and include hot flushes and leg cramps (201)**.** Raloxifene modulates bone cell homeostasis *in vitro* through actions on the proliferation and activity of osteoclasts and osteoblasts. In postmenopausal women and patients with osteoporosis, raloxifene treatment significantly reduced serum and urinary markers of bone turnover. In randomized, double-blind, placebo-controlled studies (195, 202) in postmenopausal women with osteoporosis, it was observed that raloxifene administration consistently increased lumbar spine, femoral neck, total hip and total body bone mineral density. It also reduced vertebral frature risk (203).

Conclusions

The TAE pharmacophore forms the structural core of most of the nonsteroidal estrogen antagonists. Other pharmacophores have also resulted in molecules active as estrogen antagonists, but close study of such molecules would reveal their resemblance to TAE in interacting with the ER, which would explain their similar mode of action. Therefore, a careful modification of the TAE pharmacophore as the basic residue in acyclic and cyclic structures may allow the design of new estrogen agonists

and antagonists with the desirable pharmacological activity.

Note

More information about products containing the TAE pharmacophore may be accessed by performing a structure-based search in the Prous Science Integrity® database. More than 50 additional structures (other than those referred to in this review) are retrieved under Biological Testing, Preclinical and Clinical Studies.

Acknowledgements

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