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## EM-652 (SCH57068), a pure SERM having complete antiestrogenic activity in the mammary gland and endometrium<sup>☆</sup>

Fernand Labrie<sup>a,\*</sup>, Claude Labrie<sup>a</sup>, Alain Bélanger<sup>a</sup>, Jacques Simard<sup>a</sup>,  
Vincent Giguère<sup>b</sup>, André Tremblay<sup>b</sup>, Gilles Tremblay<sup>b</sup>

<sup>a</sup> *Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL),  
2705 Laurier Boulevard, Québec City, Qué., Canada G1V 4G2*

<sup>b</sup> *Groupe de Recherches en Oncologie Moléculaire, Université McGill, Pavillon Hersey (H5),  
687 Avenue des Pins ouest Montréal, Canada H3A 1A1*

### Abstract

In order to minimize the risks of endometrial cancer and the development of resistance to antiestrogen therapy, we have synthesized the orally active antiestrogen EM-652 which is the most potent of the known antiestrogens and exerts pure antiestrogenic activity in the mammary gland and endometrium. EM-652 inhibits the AF-1 and AF-2 functions of both ER $\alpha$  and  $\beta$  while the inhibitory action of OH-TAM is limited to AF-2. EM-652, thus, inhibits Ras-induced transcriptional activity and blocks SRC-1-stimulated activity of the two receptors. The absence of blockade of AF-1 by OH-TAM could explain why resistance develops to Tamoxifen treatment. Not only the development, but also the growth of established DMBA-induced mammary carcinoma is inhibited by treatment with EM-800, the prodrug of EM-652. EM-652 is the most potent antiestrogen to inhibit the growth of human breast cancer ZR-75-1, MCF-7 and T-47D cells in vitro. When incubated with human Ishikawa endometrial carcinoma cells, EM-800 has no stimulatory effect on the estrogen-sensitive parameter alkaline phosphatase activity. When administered to ovariectomized animals, EM-800 prevents bone loss, and lowers serum cholesterol and triglyceride levels. EM-800 has shown benefits in women with breast cancer who had failed Tamoxifen. The above-summarized preclinical and clinical data clearly suggest the interest of studying this compounds in the neoadjuvant and adjuvant settings and, most importantly, for the prevention of breast and uterine cancer. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Antiestrogen; EM-652; SCH57068; Breast cancer; Uterine cancer; Chemoprevention; Osteoporosis; Estrogen receptor

### 1. Introduction

The new antiestrogens induce three-dimensional structural changes of the estrogen receptor (ER) which lead to a multitude of different activities of the ER-antiestrogen complex which are specific for each compound and for each cell type. Such ligand-induced modifications of the three-dimensional structure of ER which are unique to each antiestrogen lead, at one extreme, to a complete blockade of the physiological action of estrogens in some tissues while, in other tissues, the same ER-ligand complex completely mimics or even surpasses the natural action of estrogens.

Knowing that such an absolute tissue- or even gene-specific action of antiestrogens is possible, the objective of pharmaceutical research is to design compounds which will act in a beneficial way in all the tissues of special interest for

women's health. Breast and uterine cancer were estimated to represent 36.5% of all new cancer cases and 17.6% of all cancer deaths in women in the United States in 2000 [1] while osteoporosis and cardiovascular disease are the main causes of morbidity and mortality at postmenopause. The ideal compound would, thus, be the one having preventive as well as curative effects on all these diseases which most frequently affect women's health. What could only be a dream a few years ago has become a reality: recent discoveries of pharmaceutical research offer women the hope to achieve a marked reduction in the incidence of breast and uterine cancer while protecting against bone loss and fracture as well as reducing the risk of cardiovascular disease.

In addition to being of major importance for preventive and therapeutic medicine, the unexpected cell-specific properties of the new antiestrogens offer unique tools to investigate and further understand the detailed mechanisms of action of estrogens and antiestrogens, including the structural domains of ER responsible for their delicate interactions with the numerous other intracellular regulatory proteins (co-activators and co-repressors) which culminate

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\* Corresponding author. Tel.: +1-418-654-2704; fax: +1-418-654-2735.  
E-mail address: fernand.labrie@crchul.ulaval.ca (F. Labrie).

in cell-specific stimulation or inhibition of the expression of each estrogen-sensitive gene. The availability of these new compounds is a unique opportunity to make progress in the understanding of the detailed mechanisms involved in the numerous cellular functions regulated by estrogens under both normal and pathological conditions.

Among all risk factors, estrogens are well recognized to play the predominant role in breast cancer development and growth [2–5]. However, existing surgical or medical ablative procedures do not result in complete elimination of estrogens in women [6], due to the contribution of the adrenal glands that secrete high levels of dehydroepiandrosterone (DHEA) and DHEA-sulfate which are converted into estrogens and androgens in peripheral target tissues [7–9]. Considerable attention has, thus, focused on the development of blockers of estrogen biosynthesis and action [10–16].

Since the first step in the action of estrogens in target tissues is binding to the estrogen receptor [17,18], a logical approach for the prevention and treatment of estrogen-sensitive breast cancer is the use of antiestrogens, or compounds which block the interaction of estrogens with their specific receptor. Until very recently, however, no agent with pure antiestrogenic activity under in vivo conditions has been available.

## 2. Need of a pure antiestrogen

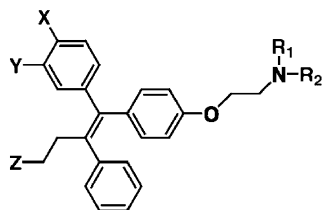
Since clinical data suggest that long-term (5 year) Tamoxifen adjuvant therapy is preferable to the short-term

(2 years) use of the antiestrogen [19,20] and studies have shown the benefits of long-term use of Tamoxifen as a chemopreventive for breast cancer [21], it has become important to develop a pure antiestrogen in order to avoid the negative effects of the partial estrogenic activity of Tamoxifen [21,22] and, thus, make available a compound having activities limited to the desired therapeutic actions. The first class of pure antiestrogens obtained were 7 $\alpha$ -substituted estradiol derivatives [10,12,14,15,23–25], especially ICI 164,384, EM-139, and ICI 182,780 (Fig. 1). However, such 7 $\alpha$ -alkyl estradiol derivatives are difficult to synthesize and their bioavailability by the oral route is very low, thus, necessitating parenteral administration.

Stimulated by the need of an improved therapy for breast cancer, considerable efforts have been devoted to the synthesis of compounds which would exert pure antiestrogenic activity in the mammary gland and uterus. As mentioned above, while Tamoxifen has beneficial effects on breast cancer, it clearly acts as an estrogen agonist in the endometrium with an increased rate of endometrial carcinoma in women taking Tamoxifen under chronic conditions [21,22]. Moreover, it is most likely that a pure antiestrogen will have beneficial effects superior to those of Tamoxifen on breast cancer prevention and treatment.

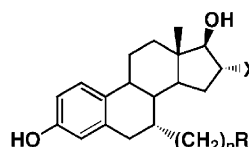
In order to meet the objective a completely tissue-specific antiestrogen, a long series of benzopyran derivatives were synthesized in our laboratory with the objective of developing an orally active compound having pure antiestrogenic activity in the mammary gland and uterus. EM-652 was, thus, the compound selected for clinical development (Fig. 1).

### A- TRIPHENYLETHYLENES



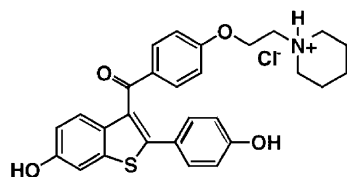
Tamoxifen	X=Y=Z=H, R <sub>1</sub> =R <sub>2</sub> =CH <sub>3</sub>
Droloxifene	X=H, Y=OH, Z=H, R <sub>1</sub> =R <sub>2</sub> =CH <sub>3</sub>
Toremifene	X=Y=H, Z=Cl, R <sub>1</sub> =R <sub>2</sub> =CH <sub>3</sub>
Idoxifene	X=I, Y=Z=H, R <sub>1</sub> , R <sub>2</sub> =C <sub>4</sub> H <sub>8</sub>

### B- STERIODALS



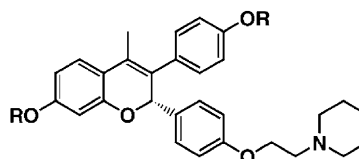
ICI 164,384	X=H, n=10, R=CON(CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>
EM-139	X=Cl, n=10, R=CON(CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>
ICI 182,780	X=H, n=9, R=SO(CH <sub>2</sub> ) <sub>3</sub> C <sub>2</sub> F <sub>5</sub>

### C- BENZOTHIOPHENES



Raloxifene

### D- BENZOPYRANS



EM-652 R=H  
EM-800 R=COC(CH<sub>3</sub>)<sub>3</sub>

Fig. 1. Molecular structures of antiestrogens.

### 3. Characteristics of EM-652 (SCH57068)

#### 3.1. Binding affinity

It can be seen in Fig. 2 that EM-652 is seven–eight-fold more potent than E<sub>2</sub> and ICI 182780 in displacing [3H]E<sub>2</sub> from the rat uterine estrogen receptor (IC<sub>50</sub> values of 0.52, 4.13, and 3.59 nM for EM-652, E<sub>2</sub>, and ICI 182780, respectively). ICI 164384 and Droloxifene are 21-fold less potent than EM-652 while Toremifene is 400 times less potent than EM-652.

#### 3.2. EM-652 blocks both AF-1 and AF-2 functions of ER $\alpha$ and $\beta$

The two ERs share many functional characteristics based on their well conserved molecular structure. AF-2 is responsible for estrogen-dependent activation through recruitment of co-activator proteins including members of the steroid receptor co-activator (SRC) family [26–33]. On the other hand, AF-1 activity is constitutive and ligand-independent [34–36].

In addition to the classical hormone activation pathway, a number of steroid receptors including ER $\alpha$  and  $\beta$  have been shown to be activated by non steroidal agents (Fig. 3) including dopamine, growth factors and PKA activators [37–42].

##### 3.2.1. EM-652 inhibits Ras-induced transcriptional activity of ER $\alpha$ and $\beta$

Potential phosphorylation of serine 118 in human ER $\alpha$  [39,43,44] and serine 60 in mouse ER $\beta$  [42] through activation of the Ras-MAPK pathway has been shown to further maximize the E<sub>2</sub> response of both estrogen receptors.

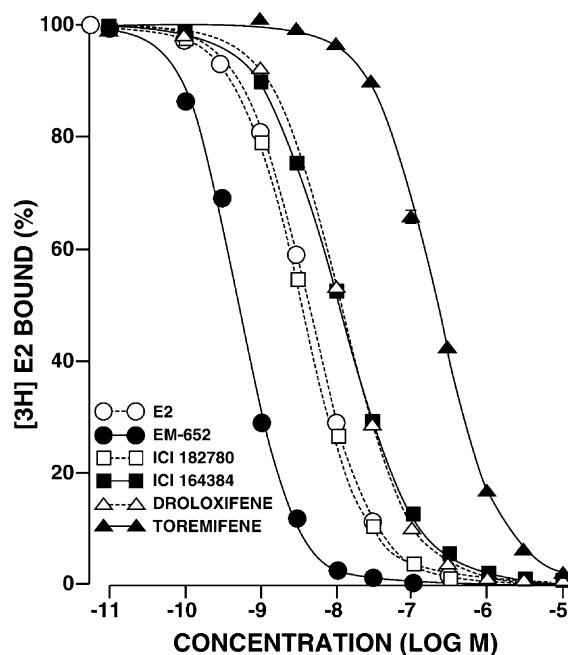


Fig. 2. Effect of increasing concentrations of EM-652, E<sub>2</sub>, ICI 182780, Droloxifene, ICI 164384, and Toremifene on [3H] 17 $\beta$ -estradiol binding to the rat uterine estrogen receptor. The incubation was performed with 5 nM [3H] 17 $\beta$ -estradiol (E<sub>2</sub>) for 2 h at room temperature in the presence or absence of the indicated concentrations of unlabeled compounds [110].

To investigate whether EM-652 could efficiently block this effect, we used the wild-type H-Ras and its dominant active form H-Ras<sup>V12</sup> in our transfection experiments, as indicated in Fig. 4. As observed previously [42,44], the addition of H-Ras contributed to increase the activity of ER $\alpha$  in the presence of E<sub>2</sub>, with an even stronger response when

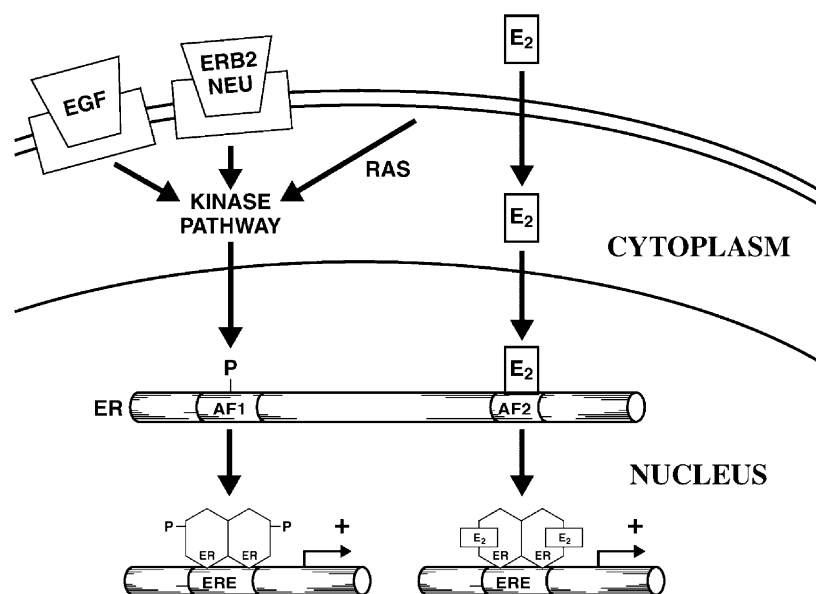


Fig. 3. Schematic representation of the dual activation mechanisms of ER by the AF-1 and AF-2 sites.

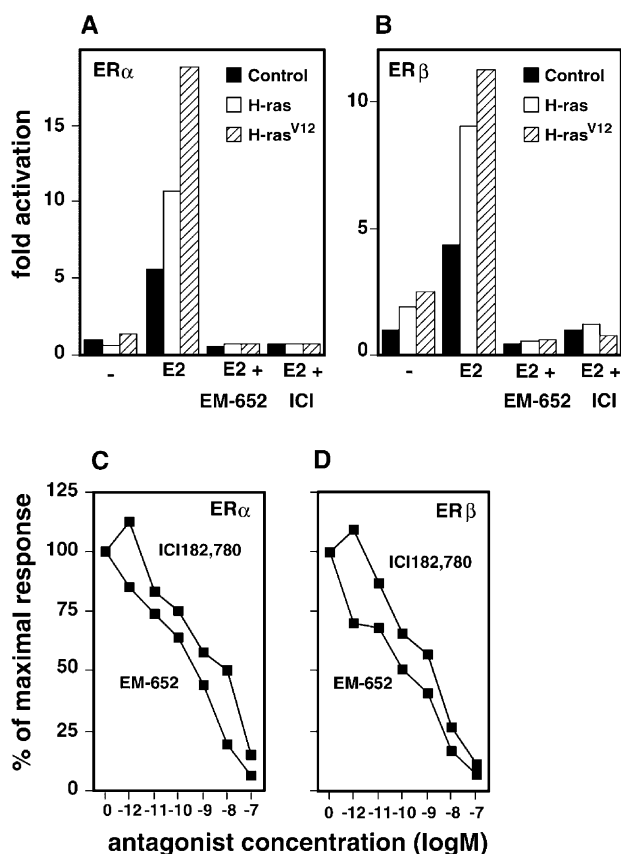


Fig. 4. EM-652 blocks the Ras-induced ER $\alpha$  and  $\beta$  transcriptional activity. (A) COS-1 cells were cotransfected with 1  $\mu$ g vitA<sub>2</sub>ERETKLuc and 500 ng pCMX-ER $\alpha$  in the presence or absence of 1  $\mu$ g H-Ras or H-Ras<sup>V12</sup> expression plasmids. The cells were then grown in the presence or absence of 10 nM E<sub>2</sub> or 100 nM of EM-652 or ICI 182,780 (ICI). The basal activity of ER $\alpha$  in the absence of estradiol was set arbitrarily at 1.0. (B) Same as in (A), except that ER $\beta$  expression vector was used. (C) Dose responses of EM-652 (filled squares) and ICI 182,780 (open squares) in the presence of 10 nM E<sub>2</sub> on ER $\alpha$  activity in COS-1 cells transfected with vitA<sub>2</sub>ERETKLuc reporter and H-Ras<sup>V12</sup> expression plasmid. The maximal induction by E<sub>2</sub> alone was set arbitrarily at 100%. (D) Same as in (C) except that ER $\beta$  expression vector was used [111].

H-Ras<sup>V12</sup> was used (Fig. 4A). These inductions by both Ras forms were completely abolished with the addition of EM-652 in the medium, as with ICI 182,780, thus, suggesting that EM-652 is effective in blocking the AF-1 activity of ER $\alpha$ . The same experiment was also conducted on ER $\beta$  where H-Ras and H-Ras<sup>V12</sup> augmented the E<sub>2</sub> response in a similar manner (Fig. 4B). Again, EM-652 and ICI 182,780 abolished the Ras effect on ER $\beta$  in the presence of E<sub>2</sub>. Interestingly, we observed a ligand-independent effect of Ras on ER $\beta$  basal activity where a two–three-fold induction occurred with H-Ras<sup>V12</sup> (Fig. 4B). On the other hand, no effect of Ras was seen on basal levels of ER $\alpha$ . The Ras induction of unliganded ER $\beta$  was blocked by EM-652 and ICI 182,780 (data not shown).

We were also interested to test whether EM-652 was efficient in blocking ER responsiveness on a natural promoter. The pS2 promoter has been extensively studied in respect to its ER $\alpha$  mediated regulation [45]. We previously showed that ER $\beta$  can also modulate transactivation of a reporter gene driven by the pS2 promoter in HeLa cells, and that the E<sub>2</sub> response was potentiated by H-Ras [42]. The effects of Ras on liganded ER $\alpha$  and  $\beta$  activities are completely abrogated by EM-652 (data not shown). Dose response analyses were also performed to further evaluate the potency of EM-652 to inhibit the effect of Ras on ER activities in the presence of E<sub>2</sub>. EM-652 was slightly more effective than ICI 182,780 in blocking H-Ras<sup>V12</sup> inductions of ER $\alpha$  and  $\beta$ , especially at lower concentrations (Fig. 4C and D).

Interestingly, both Ras and Ras<sup>V12</sup> induced the activation of transcription of ER $\beta$  in the absence of E<sub>2</sub>. Such ligand-independent activation of Ras was not observed with ER $\alpha$  [44], although it was reported with EGF treatment [39]. A similar pattern of activation of ER $\beta$ , but not ER $\alpha$ , was also observed with SRC-1. Our previous work [42] has shown that the SRC-1-induced ligand-independent activation of ER $\beta$  was not blocked by OHT, which exerts an inhibition of ER limited to AF-2 [45], suggesting that SRC-1 might interact with other regions of the receptor. A possible target region for such an interaction might be contained within the amino-terminal region of ER $\beta$  as ICI 182,780 and EM-652 inhibit the ligand-independent effect of Ras and SRC-1.

### 3.3. Inhibition of the development and growth of DMBA-induced mammary tumors in the rat

#### 3.3.1. Prevention of estrone-stimulated development of dimethylbenz(a)-anthracene-induced mammary carcinoma in the rat

7,12-Dimethylbenz(a) anthracene (DMBA)-induced mammary carcinoma in the rat is a widely used animal model to study the factors which control hormone-sensitive breast cancer in women. In fact, the development and growth of these tumors are particularly sensitive to the stimulatory action of estrogen and prolactin [2,46–58].

An ideal antiestrogen should exert a highly potent inhibitory effect on breast cancer without showing any adverse effects on the endometrium or serum lipids and bone metabolism. We have, thus, investigated the effect of the new pure antiestrogen, EM-800 [42,59–63], on the development of mammary carcinoma induced by DMBA and the effect of such treatment on the serum lipid profile as well as on bone mass in the female rat.

Nine months after DMBA administration, 95% of control animals had developed palpable mammary carcinoma. In contrast, treatment with increasing doses of EM-800 caused a progressive inhibition of tumor development ( $P < 0.0001$ , for both the Fisher's exact test and the logistic regression), the incidence being reduced to 60, 38, and 28%, respectively [64].

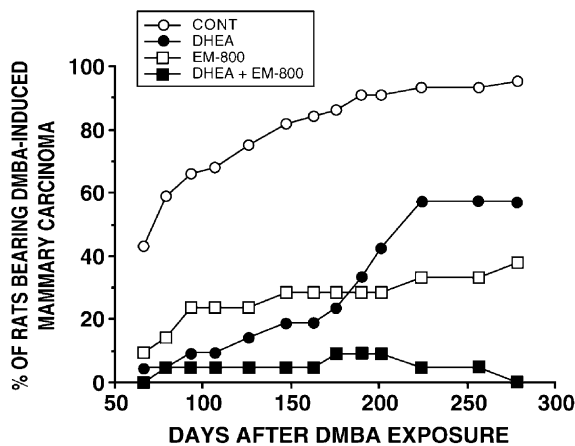


Fig. 5. Effect of treatment with DHEA (10 mg, percutaneously, once daily) or EM-800 (75  $\mu$ g, orally, once daily) alone or in combination for 9 months on the incidence of DMBA-induced mammary carcinoma in the rat throughout the 279-day observation period. Data are expressed as percentage of the total number of animals in each group [60].

### 3.3.2. Inhibition by EM-800 of estrone-stimulated growth of DMBA-induced mammary carcinoma in the rat—combination with DHEA

Since antiestrogens [11,13,65–67] as well as DHEA [58], independently, can inhibit the development of DMBA-induced mammary carcinoma, we have studied the potential benefits of combining the new antiestrogen EM-800 and DHEA on the development of mammary carcinoma induced by DMBA in the rat.

As illustrated in Fig. 5, 95% of control animals developed palpable mammary tumors by 279 days after DMBA administration. Treatment with DHEA or EM-800 partially prevented the development of DMBA-induced mammary carcinoma and the incidence was, thus, reduced to 57% ( $P < 0.01$ ) and 38% ( $P < 0.01$ ), respectively. Interestingly, combination of the two compounds led to a significantly higher inhibitory effect than those achieved by each compound alone ( $P < 0.01$  versus DHEA or EM-800 alone). In fact, the only two tumors which developed in the group of animals treated with both compounds disappeared before the end of the experiment [60].

### 3.4. Inhibition of the growth of human breast cancer cells in vitro

#### 3.4.1. Comparison of the effects of EM-652 with those of ICI 164384, OH-Tamoxifen, Tamoxifen, Droloxifene, Toremifene, Idoxifene, Raloxifene and LY353381 on basal and $E_2$ -induced cell proliferation in the MCF-7 human breast cancer cell line

We have next compared the effect of increasing concentrations of EM-652 with those of OH-Tamoxifen, Tamoxifen, ICI 164384, Droloxifene, Toremifene, Idoxifene as well as with those of Raloxifene and its related compound LY353381, on basal and  $E_2$ -induced cell proliferation in MCF-7 cells. The 8.43-fold increase in MCF-7 cell prolifera-

tion induced by a 9-day incubation with 0.1 nM  $E_2$  was competitively blocked by a simultaneous exposure to EM-800, EM-652, OH-Tamoxifen or ICI 164384 at respective  $IC_{50}$  values of 0.582, 0.321, 1.06 and 3.49 nM [63]. However, such an incubation with 50 and 100 nM Tamoxifen was only able to reverse by  $\sim 20\%$  the  $E_2$ -induced MCF-7 cell proliferation. We have also observed that after a 9-day incubation with increasing concentrations of EM-652 or EM-800 in the absence of  $E_2$ , the basal proliferation of MCF-7 cells was not affected significantly [63]. OH-Tamoxifen, on the other hand, at the concentrations of 0.1 to 10 nM caused 35–55% ( $P < 0.01$ ) stimulation of cell proliferation in the absence of  $E_2$  [63].

After 8 days of treatment of MCF-7 cells in the absence of  $E_2$ , OH-Tamoxifen, Droloxifene, and Toremifene all led to a 75–100% increase in cell proliferation which was dose-dependent: the stimulation by OH-Tamoxifen was observed at concentrations as low as 0.01 nM, the lowest concentration used, while Droloxifene gave the first significant effect at 1 nM and Toremifene stimulated basal MCF-7 cell proliferation at 100 nM (Fig. 6B) [63]. In the same experiment, EM-652, OH-Tamoxifen, Droloxifene and Toremifene inhibited the proliferative action of  $E_2$  at respective  $IC_{50}$  values of 0.19, 0.73, 30.58, and  $>500$  nM.

It can also be seen in Fig. 6A that the marked increase in MCF-7 cell proliferation induced by a 7-day incubation with 0.1 nM  $E_2$  was competitively blocked by a simultaneous exposure to EM-652, LY353381 or Raloxifene at respective  $IC_{50}$  values of  $0.23 \pm 0.02$ ,  $0.77 \pm 0.09$  and  $1.07 \pm 0.12$  whereas as illustrated in Fig. 6C, the antagonistic activity of EM-652 or Idoxifene was exerted at respective  $IC_{50}$  values and  $0.49 \pm 0.08$  and  $58 \pm 12.8$ , respectively. It can also be seen in Fig. 6A and C that, here again, the basal proliferation of MCF-7 cells was not affected by EM-652, whereas incubation with 0.02 nM LY353381, 0.05 nM Raloxifene or 2 nM Idoxifene increased significantly this estrogen-sensitive parameter by 120, 63, and 70%, respectively (all  $P < 0.01$ ).

### 3.5. Inhibition by EM-800 of the stimulatory effect of Tamoxifen on the growth of human breast cancer xenografts in nude mice

Tamoxifen has shown important benefits in breast cancer and has become the standard therapy at all stages of the disease. Although 30–50% of the patients with advanced breast cancer show a positive response to Tamoxifen, the duration of response is usually limited to 12–18 months with the development of resistance to further treatment with this antiestrogen [68–70]. As mentioned above and demonstrated in a series of studies with human breast cancer cell lines in vitro and in vivo [71–76] and supported by clinical observations [77–82], it seems reasonable to suggest that the loss of positive response to Tamoxifen treatment in breast cancer patients could be, at least in part, due to the intrinsic estrogenic activity of the compound, and its lack of blockade at the AF-1 site of ERs. This explanation is supported by

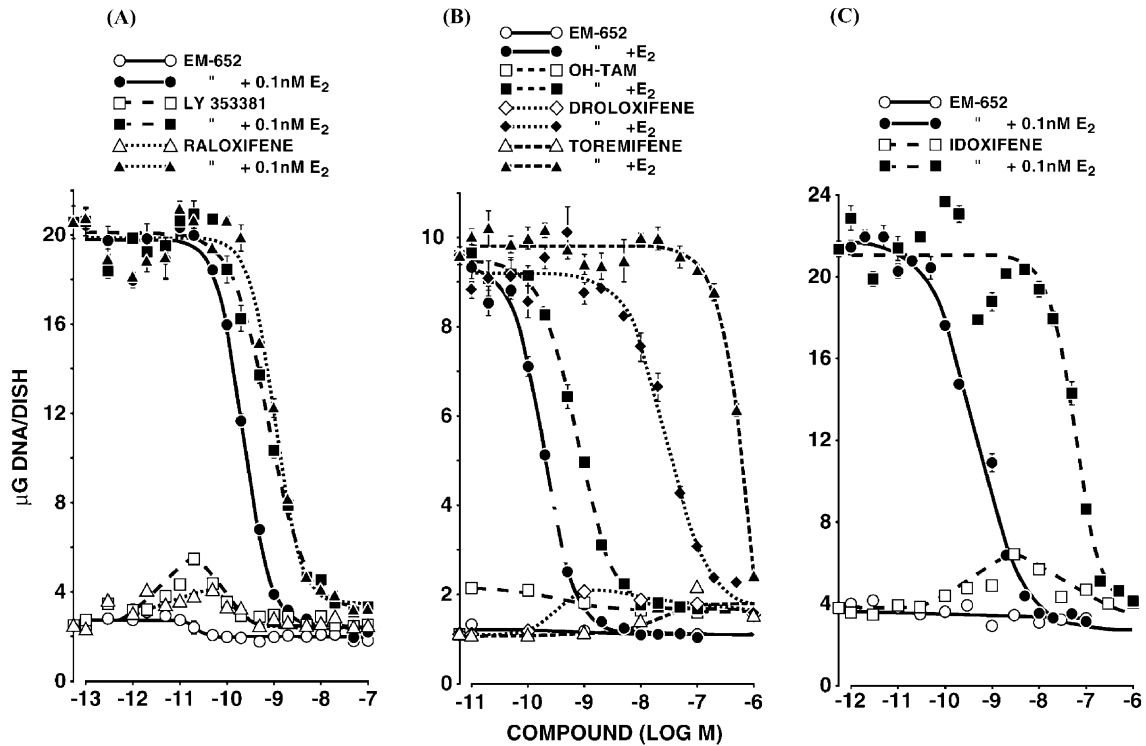


Fig. 6. Effect of increasing concentrations of EM-652, LY 353381, Raloxifene, OH-Tamoxifene, Droloxifene, Toremifene or Idoxifene on basal and E<sub>2</sub>-induced cell proliferation in MCF-7 human breast cancer cells. Three days after plating, cells were exposed for 7 (panel A and C) or 8 (panel B) days to the indicated concentrations of compounds in the presence or absence of 0.1 nM E<sub>2</sub>. Media were changed at 2- or 3-day intervals.

the finding that human breast cancer cell lines showing resistance to Tamoxifen retain their sensitivity to specific antiestrogens in vitro [76,83–85] as well as in vivo in nude mice [75,86,87].

Since human breast carcinoma xenografts in nude mice are the closest available model of human breast cancer, we have compared the effect of EM-800 and Tamoxifen alone and in combination on the growth of ZR-75-1 breast cancer xenografts in nude mice.

An example of the direct stimulatory effect of Tamoxifen on the growth of human breast cancer can be seen on Fig. 7. In fact, at 161 days, the daily oral administration of 200  $\mu\text{g}$  of Tamoxifen caused a five-fold stimulation of size of the ZR-75-1 human breast cancer xenografts compared to ovariectomy while EM-652-HCl, in agreement with its pure antiestrogenic activity in the mammary gland had no stimulatory effect. That the stimulatory effect of Tamoxifen on tumor growth is an estrogenic effect is demonstrated in the same experiment by the observation of the complete reversal of the stimulatory effect of Tamoxifen by simultaneous administration of the pure antiestrogen EM-652-HCl.

The stimulatory effect of Tamoxifen or OH-Tamoxifen on human breast cancer cell growth has been reported previously by many laboratories under in vitro [71,72,88–97] as well as in vivo [74] conditions. Such intrinsic estrogenic activity of Tamoxifen is likely to limit its success in the treatment of breast cancer in women [98].

### 3.6. Pure antiestrogenic activity of EM-652 and EM-800 in human endometrial adenocarcinoma Ishikawa cells

Since data suggest that continuous long-term Tamoxifen therapy is preferable to its usual short-term use [99] and studies on the long-term administration of Tamoxifen or Raloxifene to prevent breast cancer have been performed [21,100], it becomes important to make available a pure antiestrogen which, due to its lack of estrogenic activity, should theoretically be more efficient than Tamoxifen to treat breast cancer while simultaneously eliminating the excess risk of developing uterine carcinoma during its long-term use [21,22]. We have, thus, compared the effect of EM-800 or its active metabolite EM-652 with those of OH-Tamoxifen, OH-Toremifene, Droloxifene, Idoxifene, ICI-182780, Raloxifene and its analog LY 353381 on estrogen-sensitive alkaline phosphatase (AP) activity in human endometrial carcinoma Ishikawa cells. AP activity is well known to be stimulated by estrogens, while the other steroids, namely, androgens, progestins, mineralocorticoids or glucocorticoids, have no effect on this parameter [101].

Direct comparison of the estrogen-like activity of these mixed agonist/antagonist compounds can best be seen in Fig. 8. Incubation with the indicated concentrations of LY353381, Raloxifene, OH-Tamoxifen, OH-Toremifene, Droloxifene, or Idoxifene increased AP activity by

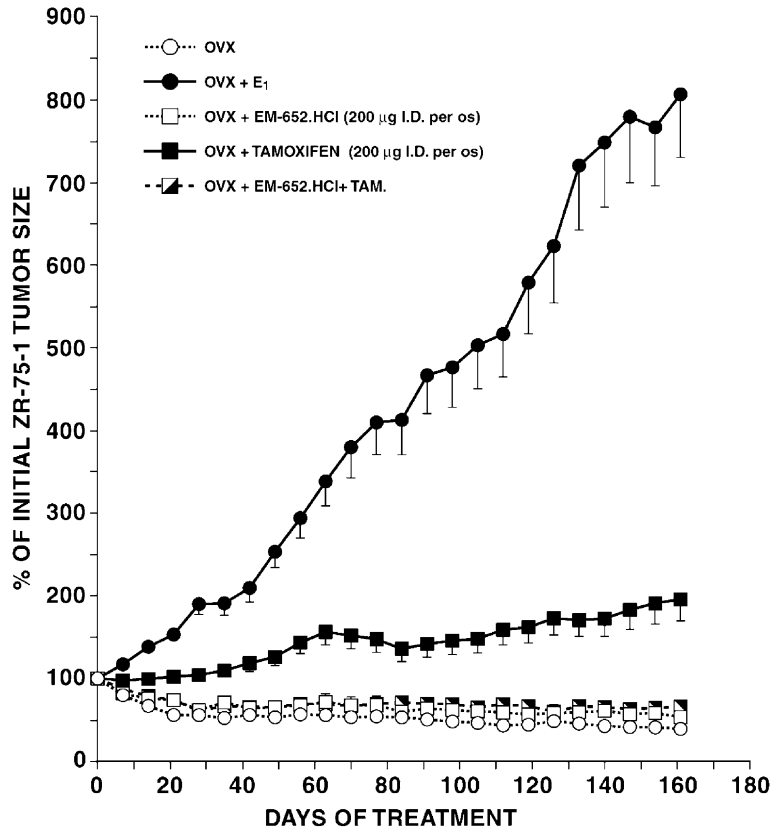


Fig. 7. Effect of daily administration of EM-652-HCl or Tamoxifen alone or in combination for 161 days on the growth of human ZR-75-1 breast tumors (xenografts) in ovariectomized nude mice. The compounds were administered orally once daily at the dose of 200 µg per mouse. Mean tumor size in ovariectomized mice receiving the vehicle alone is shown for reference. Tumor size is expressed as percent of the pretreatment value (means ± S.E.M. of 18–30 tumors per group).

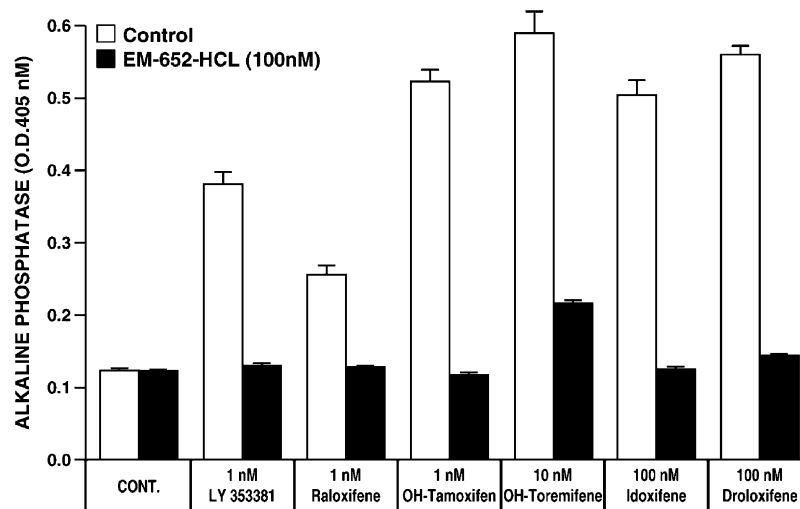


Fig. 8. Blockade by EM-652 of the stimulatory effect of LY353381, Raloxifene, OH-Tamoxifen, OH-Toremifene, Idoxifene and Droloxifene on alkaline phosphatase activity in human Ishikawa carcinoma cells. Alkaline phosphatase activity was measured after a 5-day exposure to the indicated concentrations of the specified compounds in the presence or absence of 100 nM EM-652-HCl. The data are expressed as the means ± S.E.M. of four wells with the exception of the control groups where data are obtained from eight wells.

3.1-, 2.1-, 4.3-, 4.8-, 4.0- and 4.6-fold, respectively. The blockade of the stimulatory effect of all these compounds on AP activity by simultaneous exposure to EM-652-HCl well supports the suggestion that their stimulatory effect on this estrogen-sensitive parameter in human endometrial carcinoma is mediated through the estrogen receptor as previously reported [102].

The data obtained clearly demonstrate that the novel nonsteroidal antiestrogen EM-652 exerts pure antagonistic effects while being the most potent of the compounds tested on  $E_2$ -induced alkaline phosphatase activity in human endometrial adenocarcinoma Ishikawa cells. OH-Tamoxifen, OH-Toremifene, Droloxifene, Idoxifene and Raloxifene as well as its analog LY35381, in contrast to EM-652, exert a stimulatory effect on this estrogen-sensitive parameter, an effect which can be competitively blocked by simultaneous exposure to the antiestrogen EM-652, EM-652-HCl or EM-800, thus, well supporting the

suggestion that the stimulatory effect of these antiestrogens is mediated through activation of the estrogen receptor [102].

### 3.7. Prevention of bone loss

The ovariectomized (OVX) rat is a well recognized animal model that mimics the development of estrogen deficiency-induced osteopenia in humans. It is also a useful model to study the lipid profile of compounds [103], a close parallel being found between the effect of selective estrogen receptor modulators (SERMs) as inhibitors of serum cholesterol and prevention of bone resorption [104,105]. Increasing doses of EM-800 and Raloxifene were, thus, administered orally for 37 weeks to OVX animals and the effect of these two compounds as well as that of  $17\beta$ -estradiol ( $E_2$ ) were examined on parameters of bone physiology and serum lipids.

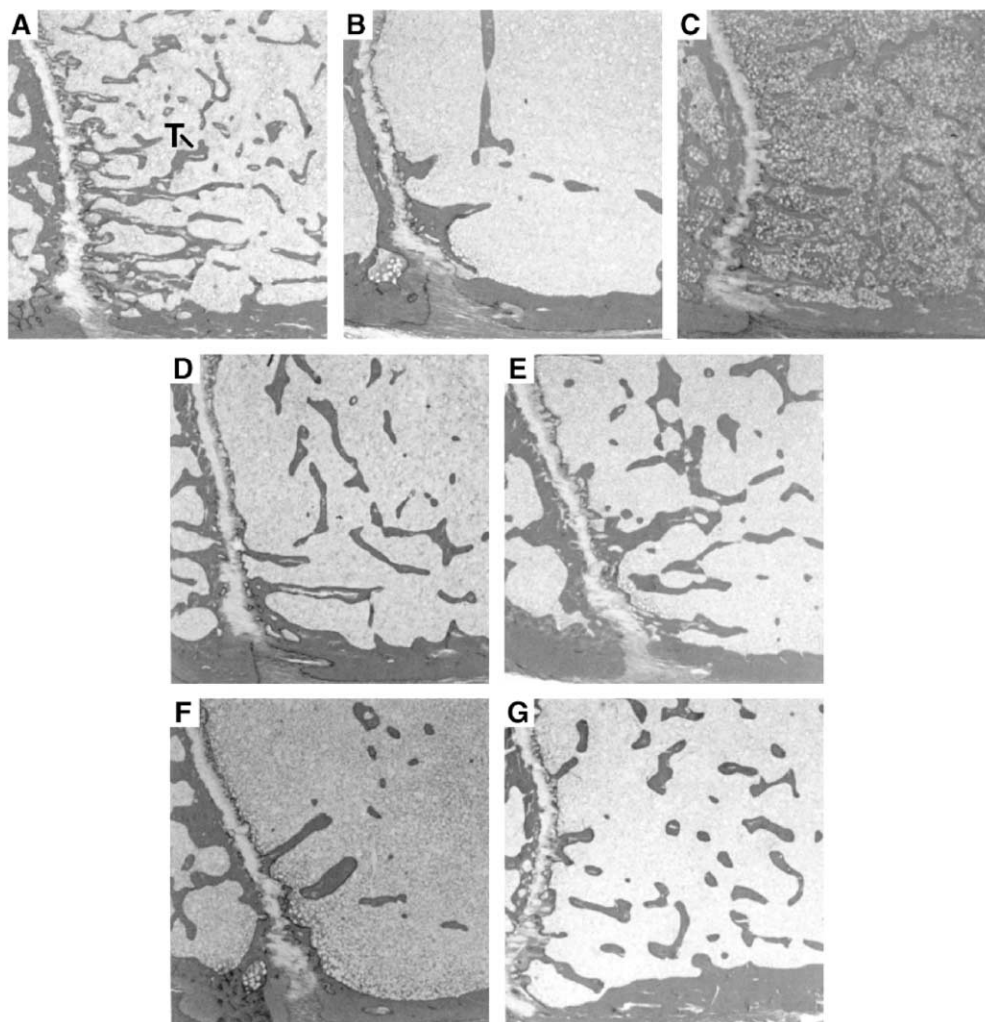


Fig. 9. Proximal tibia metaphysis from intact control (A), ovariectomized control (B), ovariectomized animals bearing an implant of  $17\beta$ -estradiol (C) and ovariectomized rats treated with 0.01 mg/kg (D) and 1 mg/kg (E) of EM-800 or 0.01 mg/kg (F) and 1 mg/kg of Raloxifene (G). Note the reduced amount of trabecular bone in ovariectomized control animals, and the significant prevention of trabecular bone volume after EM-800 (0.01 and 1 mg/kg) or Raloxifene (1 mg/kg) administration. Modified trichrome Masson–Goldner (magnification: 100 $\times$ ), T: Trabeculae [112].

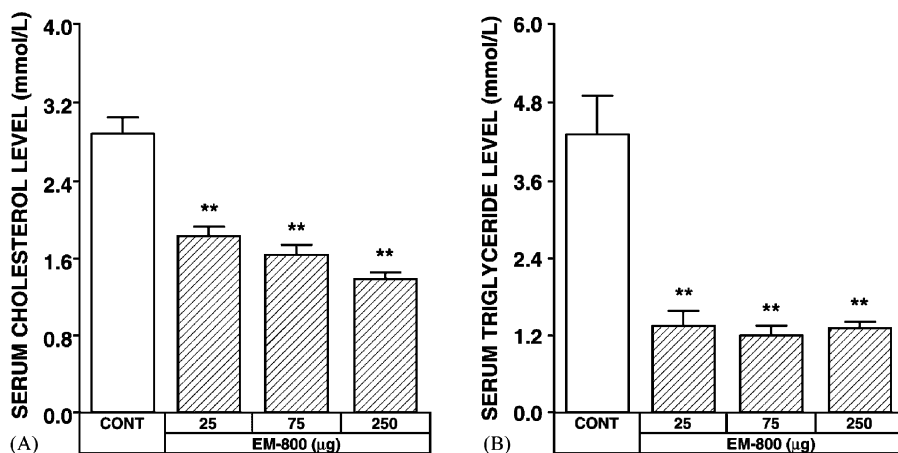


Fig. 10. Effect of daily oral administration of 25, 75, or 250 µg EM-800 for 9 months on serum cholesterol (A) and triglyceride (B) levels in the rat. The number of animals per group was 9, 14, 16, and 20, respectively. Data are expressed as the means ± S.E.M. (\*\*)*P* < 0.01 vs. control [64].

Fig. 9 illustrates the prevention of trabecular bone volume loss in the proximal tibial metaphysis induced by EM-800 and Raloxifene in OVX treated animals compared to OVX controls (B). The administration of 0.01 mg/kg of EM-800 (D) already prevented by 52% the OVX-induced osteopenia while Raloxifene had no detectable effect at the same dose (F). Treatment with 1 mg/kg of EM-800 or Raloxifene (E, G) resulted in an approximately 75% prevention of the ovariectomy-induced osteopenia.

### 3.8. Inhibitory effects of EM-652 on serum cholesterol and triglyceride levels

As can be seen in Fig. 10, a 36% reduction of serum cholesterol was already observed with the lowest dose of

EM-800 used, the serum cholesterol concentration being already decreased from  $2.9 \pm 0.18$  to  $1.8 \pm 0.09$  mmol/l at the daily 25 µg dose of EM-800 (*P* < 0.01). The daily 75 µg dose of EM-800 further decreased serum cholesterol to  $1.6 \pm 0.12$  mmol/l (*P* < 0.01) while the 250 µg dose of EM-800 caused a maximal inhibition of 52% to a value of  $1.4 \pm 0.06$  mmol/l (*P* < 0.01). The 250 µg dose had an inhibitory effect significantly (*P* < 0.01) more important than that of the 25 µg dose of EM-800, while the 75 µg dose had an intermediate inhibitory effect not significantly different from that of the 25 and 250 µg doses.

A similar inhibitory effect of EM-800 was observed on serum triglyceride levels. The daily administration of 25 µg of EM-800 for 9 months induced a near-maximal inhibition (69%) of serum triglyceride levels, which were measured at

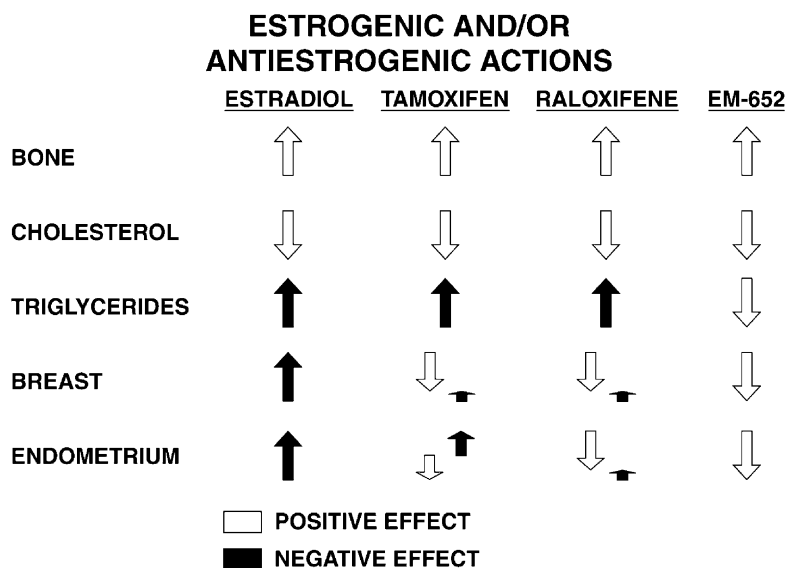


Fig. 11. Schematic representation of the estrogenic and/or antiestrogenic action of estradiol, Tamoxifen, Raloxifene and EM-652 on the main parameters important for women’s health, namely, the breast, endometrium, bone and serum lipids.

1.4 ± 0.21 mmol/l ( $P < 0.01$ ) while the value in control animals was 4.3 ± 0.62 mmol/l. Daily oral administration of 75 µg of EM-800 caused a maximal inhibition (72%) of serum triglyceride levels to 1.2 ± 0.15 ( $P < 0.01$ ) mmol/l while the 250 µg dose of EM-800 decreased serum TG levels to 1.3 ± 0.12 mmol/l ( $P < 0.01$ ). There was no statistical difference between the inhibitory effect of the three doses of EM-800.

It is, thus, of particular interest to see that EM-800 reduces both serum cholesterol and triglyceride levels, thus, indicating a potential more global beneficial effect of EM-800 on lipid metabolism. The other antiestrogens, such as Tamoxifen [83,106–108], Droloxifene [109], and Raloxifene [104], have been reported to elicit beneficial effects on the serum lipid profile, but they have not demonstrated such effect on serum triglycerides in the rat or in the human. The ability to lower both serum cholesterol and triglyceride levels seems to be unique to EM-800. The similar inhibitory effect achieved with the 25, 75 and 250 µg doses of EM-800 on serum triglyceride levels suggests a somewhat higher sensitivity of this parameter to the action of EM-800 compared to serum cholesterol.

### 3.8.1. Wide range of activities of SERMs

Fig. 11 summarizes schematically the activity characteristics of 17β-estradiol and of the three classes of antiestrogens so far available, namely, Tamoxifen (first generation SERM), Raloxifene (second generation SERM) and EM-652 (pure SERM) on the best known parameters of women's health. As can be seen in this schematic figure, Tamoxifen, while exerting beneficial effects on serum cholesterol, bone and breast cancer, possesses relatively strong estrogenic activity in the endometrium as well as some stimulatory effect on breast cancer proliferation. Raloxifene, on the other hand, represents an important improvement in terms of decrease of the stimulatory activity in the endometrium although some small estrogenic activity persists in the endometrium as well as on breast cancer cell proliferation. EM-652, on the other hand, is the only compound having pure antiestrogenic activity in the breast and endometrium while decreasing serum cholesterol and triglycerides, and protecting against bone loss.

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