(*S*)-(+)-4-[7-(2,2-Dimethyl-1-oxopropoxy)-4-methyl-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2*H*-1-benzopyran-3-yl]phenyl 2,2-Dimethylpropanoate (EM-800): A Highly Potent, Specific, and Orally Active Nonsteroidal Antiestrogen

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Breast cancer is the most frequent cancer and the second cause of cancer death in women in North America.[1] Unfortunately, the available therapies show a low rate and an usually short duration of positive responses.[2] Since estrogens are known to play a predominant role in breast cancer development and growth,[2,3] a logical approach for the treatment of estrogen-sensitive breast cancer is the use of antiestrogens which block the interaction of estrogens with their specific receptor. Despite its well-demonstrated and important clinical benefits, Tamoxifen, the antiestrogen widely available for the treatment of breast cancer, possesses mixed agonist−antagonist activities, thus potentially limiting its efficacy as blocker of estrogen action.[4] The discovery and development of specific and potent antiestrogens has thus become an important scientific challenge.

The first class of specific antiestrogens obtained were 7α-substituted estradiol derivatives,[2d,5] especially ICI 164,384, EM-139, and ICI 182,780 (Chart 1). However, the development of these compounds as drugs is problematic due to their limited oral bioavailability. We thus concentrated our efforts on the synthesis of nonsteroidal compounds having oral activity in order to overcome this difficulty. We report the synthesis of (*S*)-(+)-[4-[7-(2,2-dimethyl-1-oxopropoxy)-4-methyl-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2*H*-1-benzopyran-3-yl]-phenyl] 2,2-dimethylpropanoate [EM-800, (*S*)-**1**] and comparison of some biological properties of enantiomers (*R*)-**1** and (*S*)-**1** [or (*R*)-**6** and (*S*)-**6**].

Synthesis of chromene **6**, the racemic precursor of EM-800 [(*S*)-**1**], is shown in Scheme 1. The first step was a Friedel−Crafts reaction using $BF_3 \cdot Et_2O$ as catalyst and solvent.[6] Resorcinol was thus acylated with 4-hydroxyphenylacetic acid (**2**) to yield trihydroxydeoxybenzoin **3** at a 70% yield. The trihydroxydeoxybenzoin **3** was then protected with DHP, in the presence of TsOH as catalyst, to give the bis-THP ether **4** at a 69% yield. The Knoevenagel reaction of bis-THP ether **4** with 4-hydroxybenzaldehyde, in the presence of piperidine in refluxing benzene, gave a mixture of chromanones and chalcones at a 3:2 molar ratio. The crude intermediates were then alkylated with 1-(2-chloroethyl)piperidine monohydrochloride, in the presence of Cs_2CO_3 in refluxing acetone−water, to yield the chromanones **5** at a 65% yield.[7] The chromanones **5** were then alkylated with methyllithium, in THF at −78 °C to room temperature, to give tertiary alcohols. The crude alcohols were then dehydrated and deprotected in 90% aqueous acetic acid at 90 °C to yield the desired chromene **6** (EM-343) at a 60% yield after chromatography. The chromene **6** was an amorphous solid with variable coloration (light pink to red) and contained relatively large amounts of residual solvents (5−10% by weight).

The strategy used to obtain compound (*S*)-**1** is a chiral separation of the racemic chromene **6** and then prodrug derivatization of the active enantiomer (*S*)-**6** (EM-652). The chromene **6** was resolved using preparative HPLC and a Chiralpak AD column. Pivaloation of enantiomers (*S*)-**6** and (*R*)-**6** yielded the corresponding prodrugs EM-800 [(*S*)-**1**] and EM-776 [(*R*)-**1**], respectively, at a 80% yield. Then, in order to develop a method compatible with large-scale synthesis of (*S*)-**1** (EM-800), many asymmetric approaches were attempted without success. The reason for this difficulty could be the position of the chiral center in the molecule which is surrounded by phenoxy, phenyl, and vinyl groups. Production and/or conservation of this chiral center is difficult since the proton at this position is labile. Following a series of studies, EM-800 [(*S*)-**1**] could be obtained via the chemical resolution of racemic chromene **6** with chiral acids, despite the relatively long distance between the chiral center and the amine function. Fortunately, our chemical resolution screening program showed that (+)-CSA permits a good separation of racemate **6**. The chemical resolution of chromene **6** which leads to EM-800 [(*S*)-**1**] is outlined in Scheme 2. In brief, a solution of chromene **6** and (+)-CSA in DMF was diluted with CH_2Cl_2, thus yielding orange cubic crystals (41% yield). The crystal structure was assigned to diastereomeric salt (*S*)-**7** with 92% de.[8] Free basing of salt (*S*)-**7** with saturated K_2CO_3 gave the enantiomeric chromene (*S*)-**6** at a 84% yield. As a final step, pivaloation of compound (*S*)-**6** (EM-652) yielded the desired EM-800 [(*S*)-**1**] (80% yield, 94% ee).[9] Moreover, the mother liquor can be basified with 5% LiOH and heated at 80 °C to regenerate the racemic chromene **6** at a 92% yield.

The (*S*)- and (*R*)-enantiomers of compound **6** (EM-652 and EM-651, respectively) or their pivotal derivatives EM-800 [(*S*)-**1**] and EM-776 [(*R*)-**1**] were evaluated in *in vitro* and *in vivo* assays for their antiestrogenic activities and were compared with other known antiestrogens. The estrogen receptor affinity of compound (*S*)-**6** (EM-652), the active drug of EM-800 [(*S*)-**1**], was measured in human breast cancer and normal human uterine cytosol as described[10] (Table 1). As measured by competition studies in human breast cancer tissue, the affinity of compound (*S*)-**6** ($K_i = 0.047 \pm 0.003$ nM, RBA = 291) measured in the presence of ethanol was 2.9 and 44 times higher than that of estradiol and of the inactive enantiomer (*R*)-**6** (RBA = 6.62), respectively. Similar results were obtained on the human uterine estrogen receptor (Table 1). It can be seen in the same table that ICI 182,780 has about 10 times lower affinity than (*S*)-**6** to displace [^{3}H]E_2 from the human estrogen receptor while (*Z*)-4-OH-Tamoxifen is about 6 times less potent under the experimental conditions used. The new antiestrogen (*S*)-**6** (EM-652)

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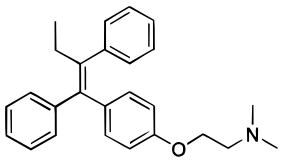
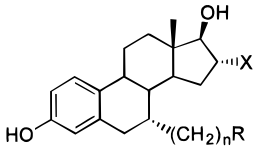
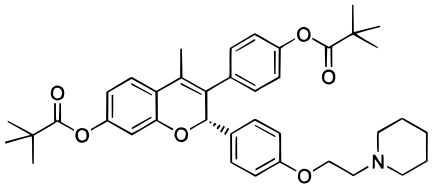
Chart 1



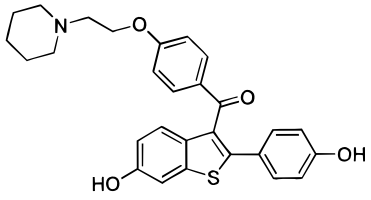
Tamoxifen



ICI 164,384 X=H, n=10, R=CON(CH$_3$)C$_4$H$_9$
EM-139 X=Cl, n=10, R=CON(CH$_3$)C$_4$H$_9$
ICI 182,780 X=H, n=9, R=SO(CH$_2$)$_3$C$_2$F$_5$

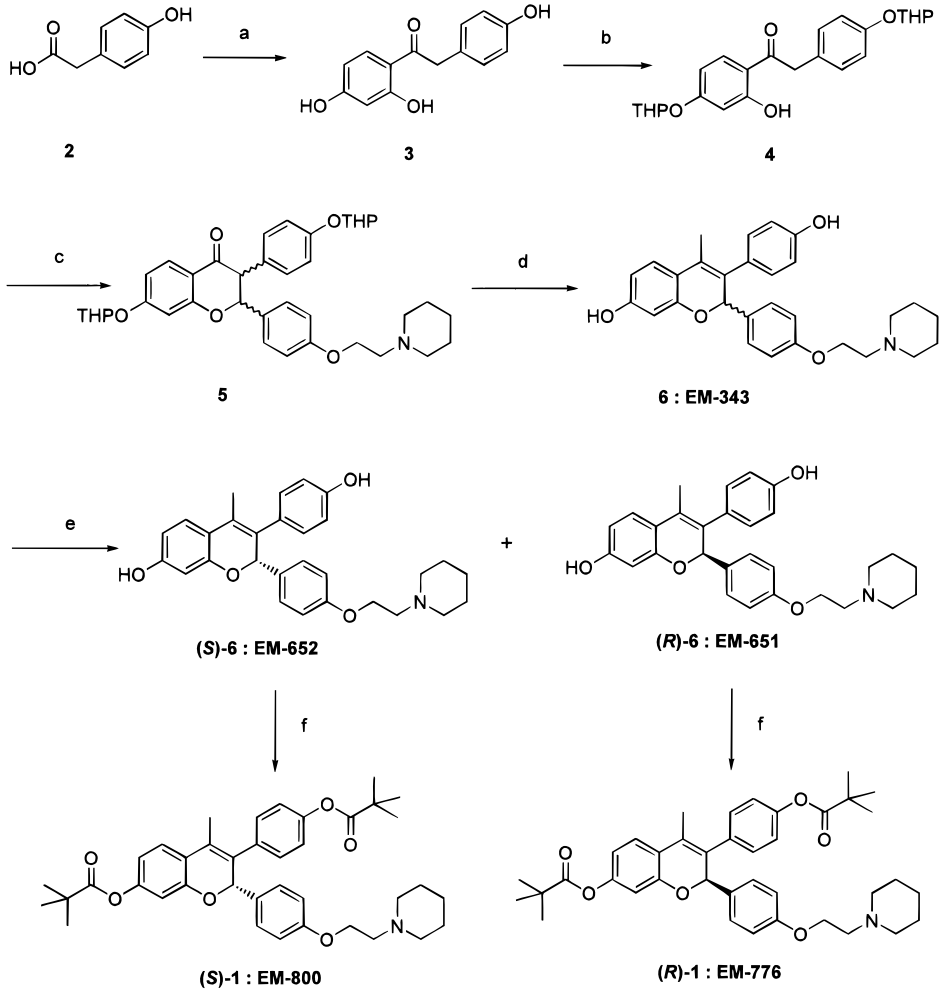


(*S*)-1
EM-800



Raloxifene

Scheme 1[a]

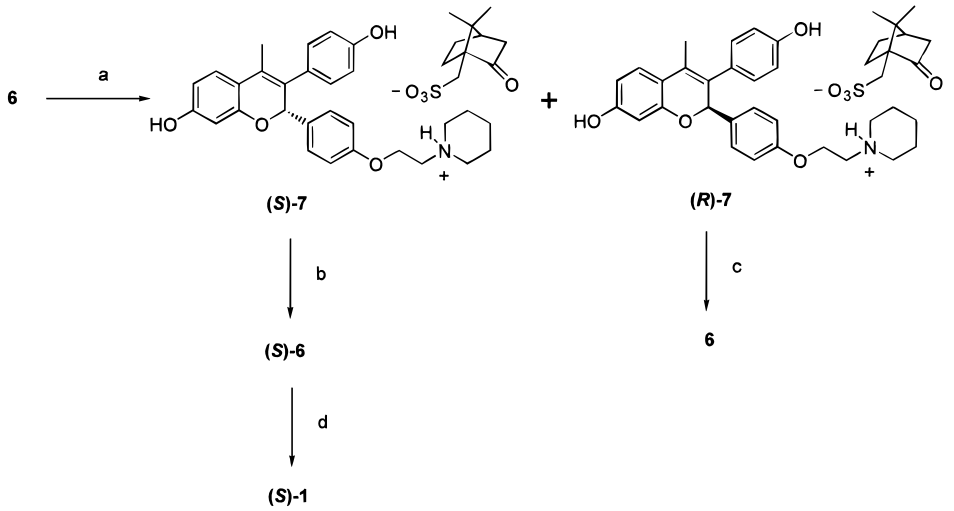


[a] Reagents and conditions: (a) resorcinol, $BF_3{\cdot}Et_2O$ (8.5 equiv), 100 °C, 1 h (70% yield); (b) DHP (9.8 equiv), TsOH (catalytic amount), 0 °C, 2.5 h (69% yield); (c) (i) 4-hydroxybenzaldehyde (1.04 equiv), piperidine (0.3 equiv), benzene, reflux, 60 h; (ii) 1-(2-chloroethyl)piperidine monohydrochloride (1.2 equiv), Cs_2CO_3 (2.4 equiv), acetone, H_2O (1.4%), reflux, 19 h (65% yield); (d) (i) MeLi (3.0 equiv), THF, −78 °C to room temperature, 3 h; (ii) AcOH, H_2O (10%), 90 °C, 0.5 h (60% yield); (e) HPLC chiral separation with a Chiralpak AD column; (f) PvCl (2.2 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 0 °C to room temperature, 2 h (80% yield).

thus shows the highest affinity for the human estrogen receptor of all the compounds tested[11] (Table 1).

The antiestrogenic activity of compounds (*S*)-**6** and (*R*)-**6** was studied on basal and estradiol-stimulated (0.1

Scheme 2[a]



[a] Reagents and conditions: (a) (+)-CSA, 2:23 DMF−CH_2Cl_2, 4 days (41% yield); (b) saturated K_2CO_3, EtOAc, 1 h (84% yield); (c) 5% LiOH, 80 °C, 3 h (92% yield); (d) PvCl (2.2 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 0 °C to room temperature, 2 h (80% yield).

Table 1. Comparison of the Estrogen Receptor Affinity of a Series of Antiestrogens and Related Compounds with Estradiol (E_2) and Diethylstilbestrol (DES) in Human Breast Cancer and Normal Human Uterine Cytosol[a]

	breast cancer				uterus			
	ethanol		DMF		ethanol		DMF	
compound	K_i (nM) (max)	RBA	K_i (max)	RBA	K_i (max)	RBA	K_i (max)	RBA
E_2	0.138	100	0.113	100	0.120	100	0.181	100
DES	0.126	110			0.128	93.5		
(*S*)-**6** (EM-652)	0.047	291	0.076	150	0.042	284	0.069	264
(*R*)-**6** (EM-651)	2.09	6.62			1.89	6.34		
(*S*)-**1** (EM-800)	4.71	2.32			11.14	1.32		
(*R*)-**1** (EM-776)	>270	<0.04						
ICI 164,384	4.60	3.00	1.53	7.46	2.33	5.15	1.76	10.3
ICI 182,780	7.63	1.81	0.755	15.1			0.668	27.2
(*Z*)-4-OH-Tamoxifen	0.249	43.8			0.346	43.8		
Tamoxifen	11.9	0.92			34.4	0.92		

[a] Incubations were performed at room temperature for 3 h using 100 μL of cytosol, 100 μL of [^{3}H]E_2 (5 nM E_2, final), and 100 μL of the indicated unlabeled compounds leading to final concentrations of 3.3% ethanol or 2.5% dimethylformamide (DMF). The apparent inhibition constant (K_i) and relative binding affinity (RBA) values were calculated as described.[10]

nM) cell proliferation in T-47D and ZR-75-1 human breast cancer cells as described.[12] In the absence of added estradiol, compounds (*R*)-**6** and (*S*)-**6** did not alter basal cell proliferation, thus showing the absence of intrinsic estrogenic activity. On the other hand, inhibition of estradiol-stimulated T-47D cell proliferation showed that compound (*S*)-**6** (IC_{50} = 0.14 ± 0.01 nM, apparent K_i value of 15 ± 1 pM) was 60 times more potent than compound (*R*)-**6** (IC_{50} = 8.4 ± 0.8 nM). Moreover, inhibition of estradiol-stimulated ZR-75-1 cell proliferation showed that compound (*S*)-**6** (IC_{50} = 0.55 ± 0.15 nM, apparent K_i = 90 ± 25 pM) was 27 times more potent than compound (*R*)-**6** (IC_{50} = 15 ± 11 nM). Furthermore, as illustrated in Figure 1A, after a 9-day incubation with increasing concentrations of (*S*)-**1** (EM-800) or (*R*)-**1** (EM-776), the 2.65-fold increase in T-47D cell proliferation induced by 0.1 nM E_2 was competitively reversed at respective IC_{50} values of 0.14 ± 0.03 and 36.2 ± 5.4 nM with apparent K_i values of 0.016 and 4.22 nM, thus showing a 260-fold higher potency of (*S*)-**1** compared to (*R*)-**1**. As observed in other studies, the new antiestrogen (*S*)-**1** and its active metabolite (*S*)-**6** have the highest potency of the antiestrogens described so far and are devoid of any stimulatory activity in human breast cancer cells *in vivo*.[13]

The (*R*)- and (*S*)-enantiomers of compound **1** were also tested for their *in vivo* effects on estrone-stimulated uterine weight in ovariectomized mice as described in ref 5f. The daily oral administration (9 days) of EM-800 [(*S*)-**1**] led to respective 17%, 55%, 77%, 84%, and 85% inhibitions of estrone-stimulated uterine weight at the 0.47, 1.6, 4.7, 16, and 47 μg doses used. Tamoxifen, on the other hand, led to 26%, 37%, 35%, 23%, and 16% inhibitions of uterine weight at equimolar doses (Table 2). It can also be seen in this table that while Tamoxifen led to a 385% increase in mouse uterine weight at the highest dose used the 54% increase observed in this experiment with (*S*)-**1** is not dose-related. This slight "delay" in the loss of wet uterine weight is an inconsistent observation.

Table 3 indicates that (*S*)-**1** (EM-800) is at least 30 times more potent than (*R*)-**1** (EM-776) to inhibit estrone-induced uterine weight. In fact, while the 1.6 μg dose of (*S*)-**1** (EM-800) already caused a 42% inhibition of estrone-stimulated uterine weight, only a 31% inhibition was observed with the 47 μg daily dose of (*R*)-**1** (EM-776). No significant trend or dose-related effect is observed when either (*S*)-**1** or (*R*)-**1** is administered alone in ovariectomized animals. Similar results were obtained with enantiomers (*S*)-**6** and (*R*)-**6**. It should be mentioned that after 6 months of treatment of intact mice with (*S*)-**1** (EM-800), uterine as well as vaginal weight were reduced below the value found in ovariectomized animals.[14b] Moreover, the endometrium

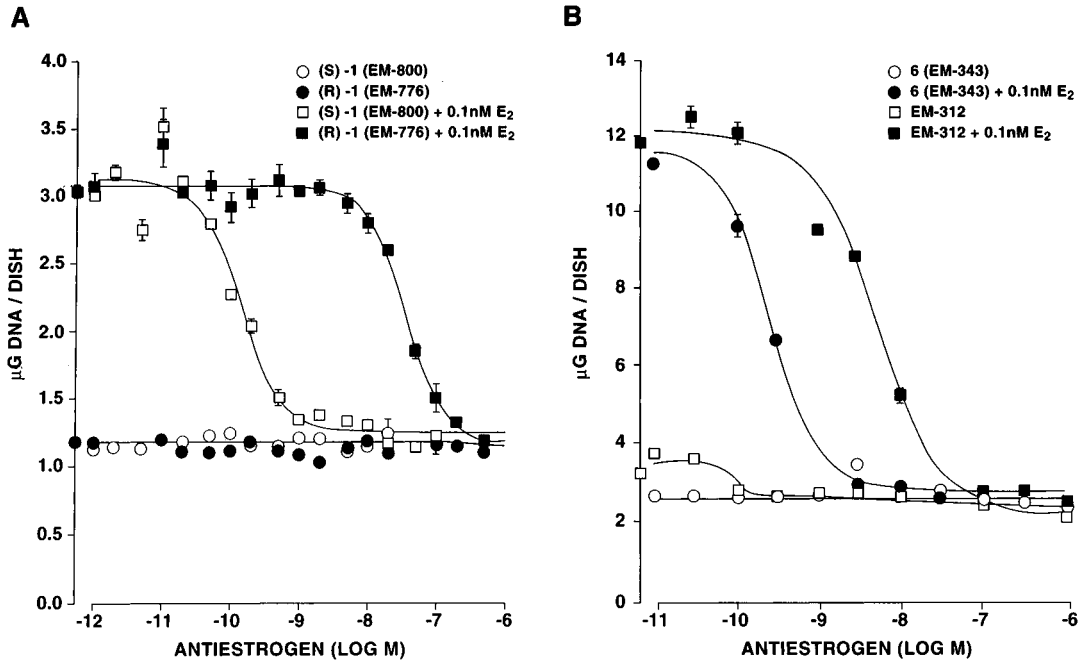


Figure 1. Comparison of the effects of increasing concentrations of (*S*)-**1** (EM-800) and (*R*)-**1** (EM-776) (panel A) as well as compounds **6** (EM-343) and EM-312 (3-(4-hydroxyphenyl)-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2*H*-1-benzopyran-7-ol)[19c] (panel B) on basal and E_2-stimulated cell proliferation in T-47D human breast cancer cells. Three days after plating, cells were exposed for 9 days to the indicated concentrations of compound in the presence or absence of 0.1 nM E_2. At the end of incubation, cell number was determined by measurement of DNA content as described.[13] The data are expressed as the means ± SEM of triplicate dishes.

Table 2. Effect on Wet Uterine Weight of 9-Day Treatment with (*S*)-**1** (EM-800) or Tamoxifen Administered Once Daily by Oral Gavage at Increasing Doses to Ovariectomized (OVX) Female Mice in the Absence or Presence of Simultaneous Treatment with Estrone (E_1, 0.06 µg, sc, twice daily)

		uterine weight (mg)[a]	
group	dose (µg)[b]	OVX + E_1	OVX
control		98.1 ± 12.5	16.2 ± 4.3
EM-800, (*S*)-**1**			
	0.47	84.0 ± 10.4*	21.0 ± 3.4*
	1.6	53.4 ± 7.5**	ND
	4.7	35.3 ± 2.9**	24.7 ± 3.8*
	16	29.2 ± 5.3**	24.0 ± 3.9*
	47	28.1 ± 3.9**	25.0 ± 4.4*
Tamoxifen			
	0.42	76.9 ± 8.5**	18.5 ± 3.5
	1.4	68.0 ± 13.0**	ND
	4.2	69.6 ± 12.5**	57.0 ± 9.7**
	14	79.0 ± 10.3**	63.3 ± 9.7**
	42	85.2 ± 6.6*	78.6 ± 12.3**

[a] ND: not determined. Data are expressed as means ± SD. *p < 0.05; **p < 0.01 vs control. [b] Equimolar doses.

Table 3. Effect on Wet Uterine Weight of 9-Day Treatment with (*S*)-**1** (EM-800) or (*R*)-**1** (EM-776) Administered Once Daily by Oral Gavage at Increasing Doses to Ovariectomized (OVX) Female Mice in the Absence or Presence of Simultaneous Treatment with Estrone (E_1, 0.06 µg, sc, twice daily)

		uterine weight (mg)[a]	
group	dose (µg)	OVX + E_1	OVX
control		81.2 ± 15.5	15.4 ± 3.8
EM-800, (*S*)-**1**			
	0.47	72.9 ± 8.1	19.0 ± 2.9
	1.6	53.4 ± 8.3**	24.1 ± 4.4*
	4.7	33.3 ± 4.2**	24.5 ± 3.2*
	16	28.9 ± 3.2**	19.4 ± 4.4
	47	31.7 ± 4.9**	21.1 ± 4.9*
EM-776, (*R*)-**1**			
	0.47	83.1 ± 10.3	16.5 ± 2.1
	1.6	88.6 ± 16.7	17.2 ± 1.7
	4.7	91.2 ± 19.6	19.8 ± 4.6*
	16	75.7 ± 17.8	15.7 ± 2.7
	47	61.1 ± 9.9**	18.0 ± 4.0

[a] Data are expressed as means ± SD. *p < 0.05; **p < 0.01 vs control.

showed histological signs of atrophy following (*S*)-**1** treatment while the endometrium was hyperplastic and hypertrophic following Tamoxifen administration. Complete inhibition of uterine weight was also observed in ovariectomized nude mice supplemented with estrone and simultaneously treated with (*S*)-**1** (EM-800) for 9 months.[14c] In agreement with the above-indicated data, compound **6** (EM-343), the racemic mixture of (*S*)-**6** and (*R*)-**6**, was found to have no stimulatory effect on uterine weight or uterine eosinophil peroxidase in ovariectomized rats.[14d] The above-indicated data support the high activity of the (*S*)-(+)-enantiomers, the high potency of the active drug (*S*)-**6** via prodrug (*S*)-**1**, and the probable absence of significant *in vivo* racemization of the compounds.

While acting as a pure estrogen antagonist in the mammary gland, endometrium, and hypothalamo-pituitary feedback in the rat, mouse, monkey, and human, EM-800 increases bone mineral density and decreases serum cholesterol and triglycerides in the rat. It is likely that such effects are mediated by mechanisms other than direct interaction of the complex ER−EM-652 with DNA.[15c] Moreover, our recent data show that EM-652 is a potent inhibitor of transactivation induced by the mouse estrogen receptors α and β.[14e]

Although interpretation of *in vivo* data in laboratory animals is limited, EM-800 [(*S*)-**1**] is the most potent antiestrogen described so far, its potency by oral administration being 2−3 times higher than that of ICI 182,780 administered subcutaneously.[14a] In fact, the

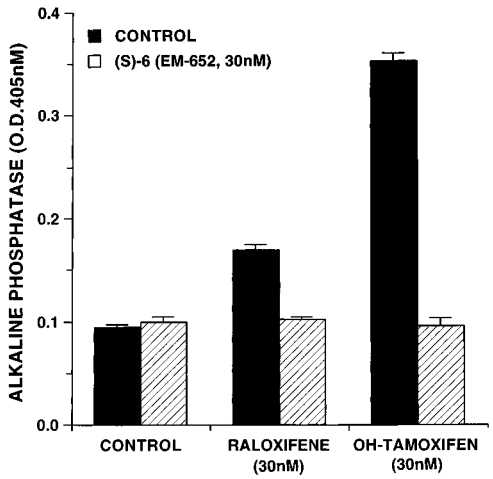


Figure 2. Blockade of the stimulatory effect of 3 nM Raloxifene or (*Z*)-4-OH-Tamoxifen on alkaline phosphatase activity by 30 nM (*S*)-**6** (EM-652) in human Ishikawa endometrial adenocarcinoma cells. Alkaline phosphatase activity was measured as described,[18] after a 5-day incubation period. The data are expressed as the means ± SD of four wells with the exception of the control groups where $n = 8$.

specificity of blockade of the estrogen receptor by antiestrogens is known to be species-, cell-, and even gene-specific,[15] thus making questionable data obtained in species other than the human and even in human tissues other than the breast and uterus when the objective is the treatment of estrogen-sensitive breast and uterine cancer. With today's knowledge, *in vivo* biological effects of antiestrogens, except possibly those on xenografts of human breast cancer in nude mice, cannot be directly extrapolated to the human but provide a useful estimate of the pharmacokinetics and metabolism of the drug.

Since a limitation to the use of Tamoxifen is its stimulatory effect on the human endometrium and the risk of inducing carcinoma,[16] compounds (*S*)-**1** and (*S*)-**6** have been studied in detail in human endometrial Ishikawa carcinoma cells.[17] To our knowledge, in human breast and uterine cancer cell lines, only the 7α-substituted estradiol derivatives such as ICI 164,384, ICI 182,780, and EM-139 and the benzopyran derivatives described above show no stimulatory effect.[13,17] In agreement with these findings indicating an estrogenic activity of all the other compounds tested, it can be seen in Figure 2 that after a 5-day incubation with 3 nM Raloxifene (Chart 1) or (*Z*)-4-OH-Tamoxifen, alkaline phosphatase activity measured as described[18] was increased by 1.8- and 3.7-fold, respectively. The complete blockade of such a stimulatory effect on alkaline phosphatase activity by simultaneous exposure to (*S*)-**6** (EM-652) supports the suggestion that the stimulatory effect of (*Z*)-4-OH-Tamoxifen and Raloxifene on this parameter is mediated through the estrogen receptor. It is also of interest to note that the stimulatory effect induced by a 6-day exposure to 1 nM E_2 on alkaline phosphatase activity in human endometrial cells was completely reversed by EM-800 [(*S*)-**1**] at an IC_{50} value of 1.3 ± 0.17 (K_i value = 0.023 nM) (Simard et al., unpublished data).

Durani and colleagues reported data on structure-activity relationships of some 2,3-diaryl-2*H*-1-benzopyrans.[19] On the basis of rat uterine estrogen receptor binding and the mouse antiuterotrophic assay, the most potent compound was 3-(4-hydroxyphenyl)-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2*H*-1-benzopyran-7-ol (EM-312). When tested in human breast cancer ZR-75-1 and T-47D cells, compound **6** was found to be 2−9 and 6−28 times more potent, respectively, than the above-indicated compound[19c] in its ability to inhibit estradiol-stimulated breast cancer cell proliferation (Simard et al., unpublished data). For example, as illustrated in Figure 1B, after a 9-day incubation with compound **6** (EM-343) or EM-312, the 3.65-fold increase in T-47D cell proliferation induced by 0.1 nM E_2 was reversed at respective IC_{50} values of 0.243 ± 0.019 nM (K_i value = 0.028 nM) and 4.6 ± 0.7 nM (K_i value = 0.525 nM), thus indicating a 19-fold higher potency of **6** (EM-343) compared to EM-312.

EM-800 [(*S*)-**1**] (or EM-652, (*S*)-**6**) shows some analogy with the 7α-substituted estradiol derivatives mentioned above. In fact, superimposition of these pseudoplanar compounds shows that the flexible chains containing a polar function (the α-side) and the hydroxyl groups (7- and 4′-positions of EM-652 [(*S*)-**6**] with 3- and 17β-positions of estradiol derivatives, respectively) are in the same region in space. Moreover, the methyl group at the 4-position of EM-800 [(*S*)-**1**] can be superimposed with the methylene group at the 11-position of the estradiol derivatives (C ring). Benzopyran-based antiestrogens also have structural analogy with diethylstilbestrol and Tamoxifen.[20]

In summary, we have synthesized a new nonsteroidal antiestrogen, EM-800 [(*S*)-**1**], which is the most potent antiestrogen described so far. The above-indicated data show that the (*S*)-(+)-enantiomer of chromene **1** (or **6**) is a much more potent antiestrogen than the (*R*)-(+)-enantiomer of chromene **1** (or **6**). The availability of such a potent and specific or pure antiestrogen for the human breast and endometrial tissues could well lead to a significant improvement in the treatment of estrogen-sensitive breast and uterine cancer as well as a series of nonmalignant estrogen-sensitive diseases. Specificity of the new antiestrogen on a series of estrogen-sensitive genes and biological responses is under investigation.

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(9) The optical rotations of compounds (*S*)-**1** and (*R*)-**1** are $[\alpha]^{24}_D$ 95° (*c* 1.0, CH_2Cl_2) and $[\alpha]^{24}_D$ −94° (*c* 1.0, CH_2Cl_2), respectively.

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