A Selective Estrogen Receptor Modulator for the Treatment of Hot Flushes

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Received September 30, 2005

Abstract: A selective estrogen receptor modulator (SERM) for the potential treatment of hot flushes is described. (*R*)-(+)-7,9-difluoro-5-[4-(2-piperidin-1-ylethoxy)phenyl]-5*H*-6-oxachrysen-2-ol, LSN2120310, potently binds ERα and ERβ and is an antagonist in MCF-7 breast adenocarcinoma and Ishikawa uterine cancer cell lines. The compound is a potent estrogen antagonist in the rat uterus. In ovariectomized rats, the compound lowers cholesterol, maintains bone mineral density, and is efficacious in a morphine dependent rat model of hot flush efficacy.

Hot flushes (flashes) are characterized by a warming sensation that begins in the chest and moves toward the neck and head and are often accompanied by sweating, palpitations, and cutaneous flushing. The episodes generally last from 30 s to 10 min. The majority of postmenopausal women will experience hot flushes, with a significant percentage of these women continuing to suffer symptoms for more than five years.[1,2] The hot flush event itself is thought to be centrally mediated, resulting from a transient lowering of the thermoregulatory set point in the hypothalamus.[3] Regulation of the thermoregulatory process may involve catecholamines, estrogen, testosterone, opioids, and serotonin, among others.[4] In fact, compounds that modulate the signaling pathways of each of these hormones/neurotransmitters have been evaluated clinically for the treatment of hot flushes. Unfortunately, all the investigated therapies suffer from poor efficacy, are associated with unacceptable side effects, or are contraindicated for certain patient populations.[3]

Despite being identified as an ailment of menopause for hundreds of years, the precise mechanism underlying the alteration of the thermoregulatory set point is not clear. The link with declining estrogen levels is widely recognized, however, and compounds that interact with the estrogen receptor can have a significant effect on the induction or alleviation of hot flushes. Tamoxifen (Figure 1) was reported to induce hot flushes in more than 50% of patients,[5] and raloxifene was also reported to increase the incidence of hot flushes in clinical trials.[6] Estrogen or hormone replacement therapy (ERT or HRT) is currently the treatment of choice and is effective in >80% of women who initiate treatment. However, estrogen replacement therapy is not recommended for women with a history of breast cancer, uterine cancer, ovarian cancer, or venous thromboembolism. Recent data also suggests HRT may not be suitable for women with coronary artery disease.[7] Perhaps because of these concerns and/or the incidence of undesirable side effects, the compliance rate for women on ERT or HRT is remarkably low.[8] The need for an improved treatment is clear. A recent report detailed a spiro indane derivative that was shown to be efficacious in preclinical models of hot flushes but had weak ER binding and weak antagonist properties in a breast cancer cell line (MCF-7).[9] In addition, it was not reported to have antagonist activity on the uterus. We have identified a compound (**1**, Figure 1) that possesses a desirable SERM profile on uterine, breast, bone, and cardiovascular parameters, while displaying potent and efficacious activity in a morphine-dependent ovariectomized (OVX) rat model of hot flush efficacy. The compound is related to a series of conformationally restricted raloxifene analogues reported by Grese et al.[10]

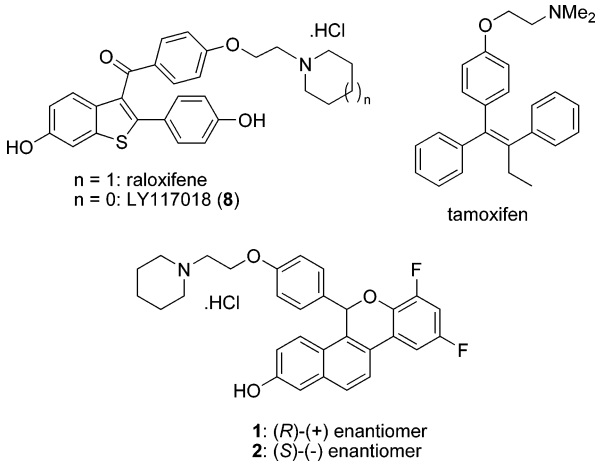

Figure 1.

Table 1 [a]

	binding		MCF7	Ishikawa agonist		Ishikawa antagonist	
	K_i ERα (nM)	K_i ERβ (nM)	IC_{50} (nM)	EC_{50} (nM)	efficacy (%)	IC_{50} (nM)	efficacy (%)
1	0.3 ± 0.1	0.5 ± 0.2	1.4 ±0.9	NC	3.2 ± 1.0	5.3 ± 1.2	100 ± 7
2	5.8 ± 0.8	54 ± 7	330 ± 100	NC	3.3 ± 1.6	430 ± 100	68 ± 33

[a] NC: not calculated.

The synthesis began with naphthalene triflate **3** (Scheme 1). To facilitate liberation of the A-ring phenol at the end of the synthesis, a protecting group transposition was undertaken. The HCl salt of **3** was first made in order to minimize ethoxypiperidine side chain cleavage during the subsequent BBr_3-mediated demethylation. Treatment of the HCl salt of triflate **3** with boron tribromide afforded a quantitative yield of phenol **4** which was then treated with MsCl and Hunig's base to afford **5** in excellent yield (99%). This versatile intermediate was then converted in situ to the corresponding neopentylglycolato boronic ester under palladium catalysis. Subsequent addition of 1-bromo-2,3,5-trifluorobenzene and additional bis(acetato)-bis-(triphenylphosphine)palladium catalyst afforded the desired coupled product. This material was then filtered and the mesyl protecting group was cleaved using KOH in MeOH at room temperature to yield phenol **6** (64% yield from **5**). Asymmetric reduction of the benzophenone carbonyl was achieved using the Corey protocol.[11] Namely, ketone **6** was treated with (*R*)-(+)-α,α-diphenyl-2-pyrrolindinemethanol and borane in THF, followed by addition of ethanolamine to decomplex the product, to afford chiral alcohol **7** in excellent yield (98%) and enantiomeric purity (99.2% ee). The final step in the synthesis involved a base-promoted intramolecular S_NAr reaction whereby

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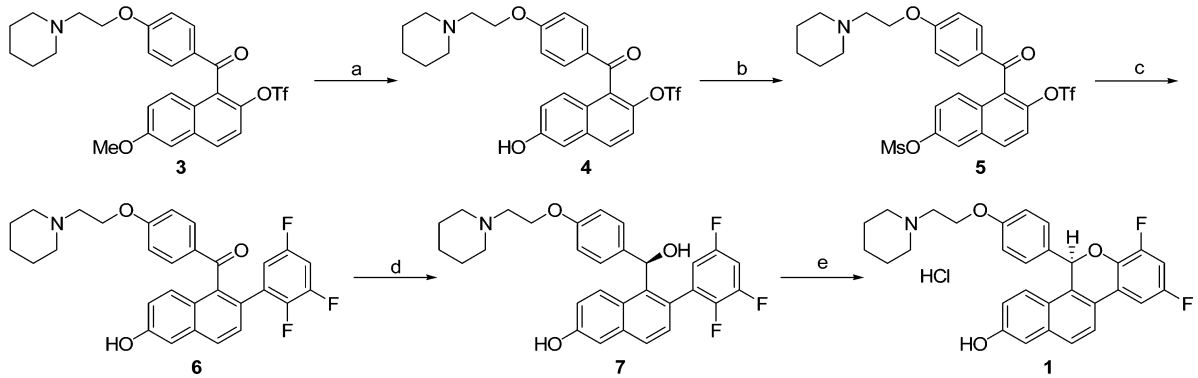
10.1021/jm0509795 CCC: $33.50
Published on Web 01/12/2006

Scheme 1[a]



[a] a. i. HCl ii. BBr_3. b. MsCl, i-Pr_2NEt. c. i. bis(neopentyl glycolato)diboron, $Pd(OAc)_2(PPh_3)_2$, CsF, ACN, 75 °C. ii. 1-bromo-2,3,5-trifluorobenzene. iii. KOH, MeOH. d. i. (*R*)-(+)-α,α-diphenyl-2-pyrrolidinemethanol, BH_3-THF, 45 °C. ii. ethanolamine e. i. KO^{t}Bu, THF, ii. HCl.

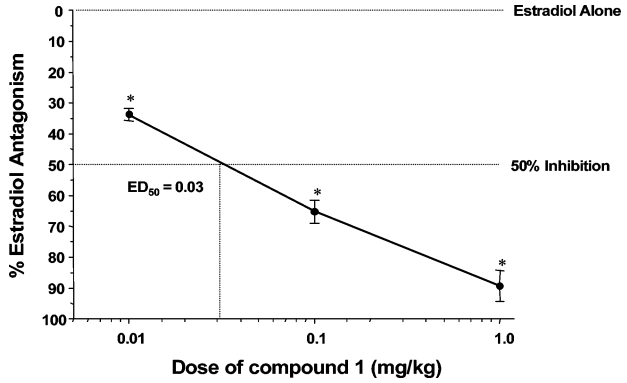


Figure 2. Three week old Sprague Dawley female rats were orally treated with estradiol (0.1 mg/kg) and 1.0, 0.1, and 0.01 mg/kg SERM for 3 days, 6 rats per group. Test compounds were dissolved in 20% *β*-hydroxy-propylcyclodextrin and administered orally 15 min prior to E2. Animals were weighed and then euthanized (by carbon dioxide asphyxiation), and the uteri were collected and weighed. The percentage inhibition of the estrogen-induced response was calculated as: $100 \times (UWR_{estrogen} - UWR_{test\ compound}/UWR_{estrogen} - UWR_{control})$ where UWR = uterine weight/body weight ratio. *Significant decrease from estradiol alone for each dose, $p < 0.05$.

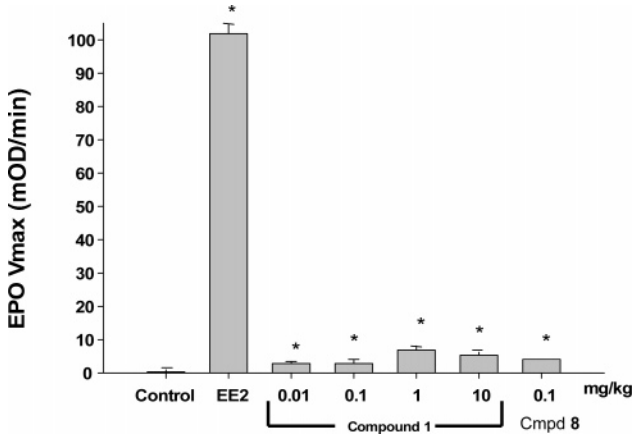


Figure 3. 75 day old SD rats were ovariectomized and were orally treated with ethinyl estradiol (EE2, 0.1 mg/kg) or compound **1** once daily starting 14 days post-ovariectomy. After 4 days of treatment, eosinophil peroxidase (EPO) activity was measured. *All groups significantly different from control, $p < 0.05$.

alcohol **7** was heated with K-OBu-t in THF to afford (*R*)-(+)-7,9-difluoro-5-[4-(2-piperidin-1-ylethoxy)phenyl]-5*H*-6-oxachrysen-2-ol, **1**, in 97% yield.[12] The (*S*) enantiomer **2** was isolated by chiral chromatography of a racemic mixture resulting from a nonasymmetric reduction of benzophenone **6** followed by cyclization.

Benzopyran **1** was initially examined for its ability to bind to the estrogen receptor using a cell free competition binding assay where displacement of ^{3}H-17*β*-estradiol by the compound was measured (Table 1).[13] The compound was tested a total of seven times and found to possess high affinity to both ERα (K_i = 0.3 ± 0.1 nM) and ER*β* (K_i = 0.5 ± 0.2 nM) and was considerably more potent than its enantiomer **2** (K_i ERα = 5.8 ± 0.8 nM; K_i ER*β* = 54 ± 7 nM).

Since the breast is highly estrogen responsive, and HRT has been implicated with an increase in the risk of breast cancer, we considered it important that desirable compounds were estrogen antagonists in mammary cells. In addition, the estrogen receptor ligand previously reported to be efficacious as a hot flush agonist possessed only very weak activity in a breast cancer proliferation assay.[9] Profiling was therefore undertaken to determine the antagonist effects of our compounds on breast adenocarcinoma-derived MCF-7 cells stimulated by 10 pM 17*β*-estradiol.[13] Compound **1** was a potent inhibitor of cell proliferation in this assay (IC_{50} = 1.4 ± 0.9 nM) and was approximately 240-fold more potent than its antipode, a trend that is consistent with the decreased binding affinity of **2**. Prior to testing the compounds in vivo for their ability to act as agonists or antagonists on the uterus, the compounds were profiled in an Ishikawa human uterine cell line. Agonist activity was determined by quantitating alkaline phosphatase induction by the compound alone, and antagonist activity was determined by inhibition of alkaline phosphatase induction by the compound in the presence of 1 nM 17*β*-estradiol.[14] Compound **1** was thus determined to be a highly potent (IC_{50} = 5.3 ± 1.2 nM) and efficacious (100 ± 7% efficacy) antagonist, while displaying weak and variable agonist activity of approximately 3.2% relative to 17*β*-estradiol.

In vivo uterine effects of the compound were assessed in an immature rat model of estrogen antagonist activity and in an ovariectomized rat model of estrogen agonist activity on uterine stimulation. In the immature (3 week old) rats, benzopyran **1** was capable of inhibiting the effects of administered estrogen at oral doses of 0.01−1 mg/kg/day for 3 days (Figure 2) and was remarkably potent in this assay (ED_{50} = 0.03 mg/kg). Conversely, compound **2** displayed no dose dependent antagonism of estradiol stimulation (data not shown), which again is consistent with the weak binding and functional profile of **2** in vitro.

In a 4-day OVX model, we measured eosinophil peroxidase (EPO) activity which is a very sensitive gauge of estrogenic activity in the uterus.[15] Activity in this model was compared to **8** (LY117018, Figure 1), an analogue of raloxifene that has been characterized extensively in vivo.[16] Compound **1** displayed

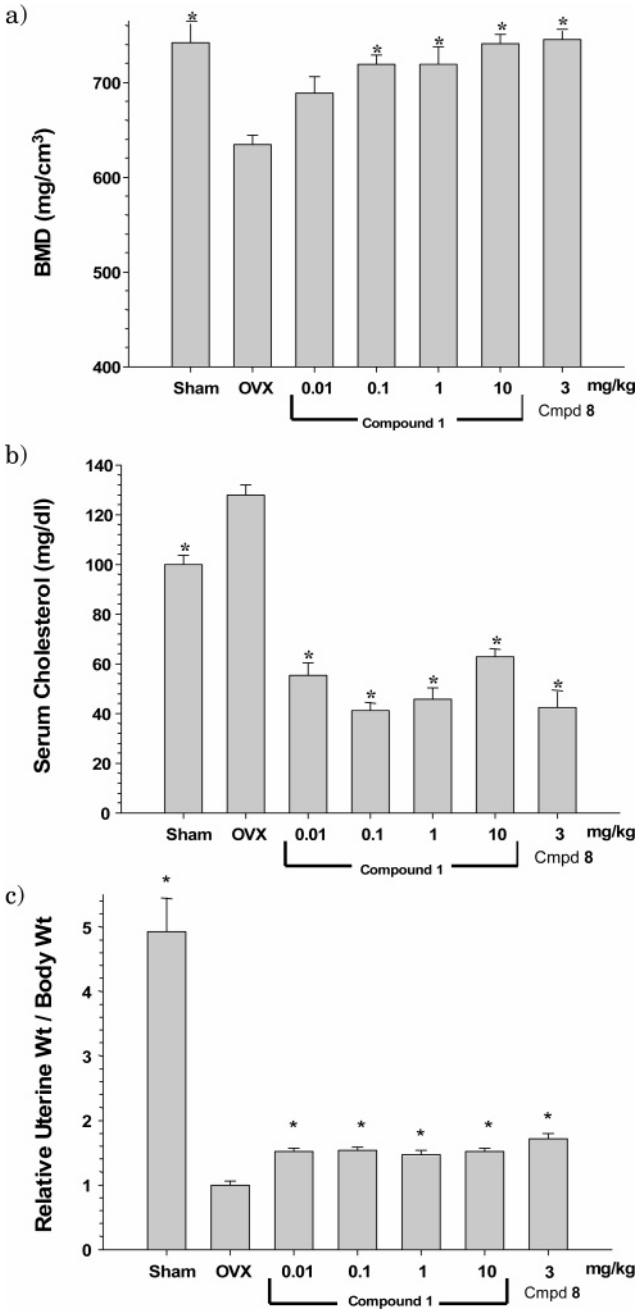


Figure 4. Virgin 6 month old SD rats were ovariectomized and were orally treated with compound once daily starting 3 days postovariectomy. After 42 days of treatment, animals were sacrificed. (a) Volumetric bone mineral density (vBMD) of the mid-transverse section of lumbar vertebra L-4 was measured using quantitative computed tomography. *Significant increase from OVX control, $p < 0.05$. (b) Serum cholesterol levels were determined by measurement of cholesterol esterase/cholesterol oxidase activity utilizing a Roche/Hitachi 917 automated chemistry analyzer. *Significant decrease from OVX control, $p < 0.05$. (c) Uteri were extracted and their wet weight was measured. *Significant increase from OVX control, $p < 0.05$.

minimal agonist effects in this assay, with slight effects on EPO activity observed and comparable to benzothiophene **8** (Figure 3). The data from the immature rat model and the 4-day OVX model is consistent with the in vitro Ishikawa data, which suggested that **1** is a potent antagonist and weak agonist in the uterus.

Bone and cardiovascular effects as well as long-term effects on the uterus were assessed in a 42 day OVX rat model of estrogen deficiency-induced osteopenia, where both bone mineral density (BMD) and cholesterol changes were measured. As can be seen in Figure 4a, compound **1** significantly maintained BMD relative to the loss seen in OVX control rats at doses as low as 0.1 mg/kg. The ability to block ovariectomy-induced bone loss was comparable to compound **8**. Compound **1** also displayed potent lowering of cholesterol in this model where just 0.01 mg/kg of compound reduced serum cholesterol by approximately 50% (Figure 4b). We also measured uterine growth response to see if long-term dosing would cause an agonist response that was not seen with the short-term dosing described above. As shown in Figure 4c, there was minimal activity on uterine wet weight, less than that seen with **8**. Thus, benzopyran **1** possesses the desired agonist profile on the skeletal and cardiovascular systems, while maintaining potent antagonist activity in reproductive tissues.

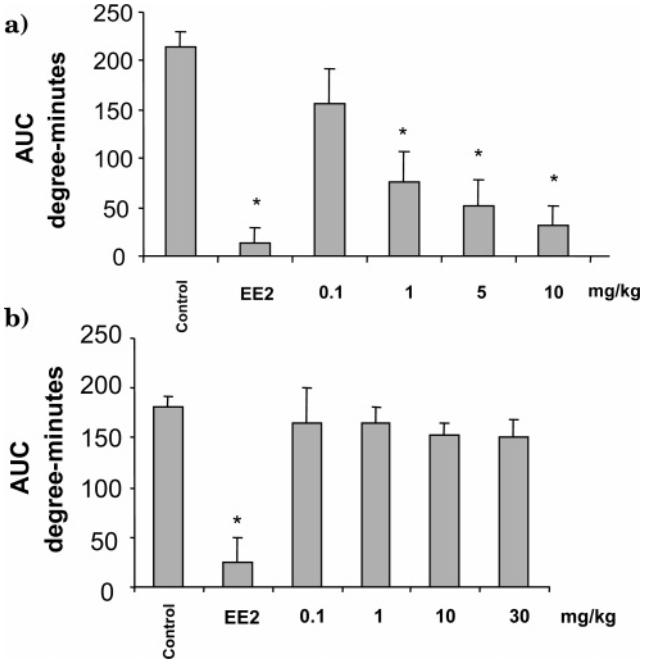


Figure 5. 60 day old SD rats were ovariectomized and were orally treated with either ethinylestradiol (EE2, 0.3 mg/kg) or test compound once daily starting on day 14 postovariectomy. On days 15 and 17, a single 75 mg morphine pellet was surgically implanted. On day 21, animals were anesthetized with ketamine (80 mg/kg, im) 2 h after the final dose of compound. Temperature-sensitive probes were applied to the dorsal side of the tail base, and temperature measurements were recorded every 15 s for a 1 h period. 45 min after ketamine administration, animals were treated with naloxone (1 mg/kg, sc), and temperature changes (AUC) were recorded for 45 min. *Significant decrease from control, $p < 0.05$. (a) Data for compound **1**. (b) Data for compound **8**.

The ability of the compound to act as a potential therapy for the treatment of hot flushes was assessed using a slight modification of the morphine dependent rat model. In this assay, estrogen, or compounds that act as estrogen agonists in the thermoregulatory centers, attenuate the increase in tail skin temperature observed on treatment of morphine dependent rats with an opioid antagonist such as naloxone. This model, or modifications thereof, has been widely used to evaluate the effect of SERMs and estrogens on vasomotor activity.[17] Compound **1** exhibited a dose-dependent response and had a significant effect on temperature at doses of 1, 5, and 10 mg/kg (Figure 5a). Conversely, raloxifene analogue **8** in an identical assay showed no effect on temperature relative to the morphine control at doses of 0.1 to 30 mg/kg (Figure 5b).

In summary, we have developed an asymmetric synthesis of (*R*)-(+)-7,9-difluoro-5-[4-(2-piperidin-1-ylethoxy)phenyl]-5*H*-

6-oxachrysen-2-ol (LSN2120310), **1**, in high yield and excellent ee from naphthalene triflate **3**. Benzopyran **1** exhibits a typical SERM profile in vitro, in that it potently binds ERα and ERβ and is an antagonist in breast (MCF-7) and uterine (Ishikawa) cancer cell lines. In vivo, compound **1** is a potent antagonist of estrogen action on the uterus in an immature rat model. In a longer term in vivo assay in ovariectomized rats, the compound increases bone mineral density, lowers serum cholesterol, and exhibits minimal uterine agonist activity. Compound **1** also displays dose-dependent activity in an ovariectomized rat model of hot flush efficacy and may have utility for the treatment of a variety of menopausal symptoms.

Acknowledgment. We thank members of the Lead Optimization Biology group for conducting ER binding, and MCF-7 and Ishikawa assays, and Ms. Julie Ruff for her assistance with the preparation of this manuscript.

Supporting Information Available: Detailed experimental procedures for the synthesis and characterization of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0509795