Novel Chromene-Derived Selective Estrogen Receptor Modulators Useful for Alleviating Hot Flushes and Vaginal Dryness

Nareshkumar Jain,* Ramesh M. Kanojia, Jiayi Xu, Guo Jian-Zhong, Emmanuel Pacia, Muh-Tsann Lai, Fuyong Du, Amy Musto, George Allan, DoWon Hahn, Scott Lundeen, and Zhihua Sui

Johnson & Johnson Pharmaceutical Research & Development LLC, 1000 Route 202, Raritan, New Jersey 08869

Received March 27, 2006

Abstract: A novel SERM (selective estrogen receptor modulators), **1**-(*R*), a chromene-derived bisbenzopyran, was discovered to alleviate hot flushes and effectively increase vaginal fluidity in rats. Moreover, **1**-(*R*) was found to have beneficial effects on plasma cholesterol and bone metabolism while maintaining antiestrogenic activity in the uterus. The biological profile of its enantiomer **1**-(*S*) was also evaluated.

The endogenous steroid estrogen is a critical mediator of many physiological functions related to development, growth, and maintenance of a large number of tissues in both females and males. Selective estrogen receptor modulators (SERM[a]) are compounds that act as estrogen agonists on selected targets while being estrogen antagonists on others.[1] Hot flushes and vaginal dryness are the most common symptoms associated with menopause in women. A hot flush is characterized by the sudden onset of a hot feeling, sweating, palpitation, and anxiety and affects approximately 75% of women as they enter into menopause.[2] More than 50% postmenopausal women experience lack of vaginal lubrication that leads to several genital complaints associated with a diminished frequency of all forms of sexual behavior.[3] Indeed, hormone therapy (HT) alleviates both of these symptoms in 80−90% of women and has been recognized as the most effective treatment for hot flushes and vaginal dryness.[4] Despite the effectiveness of HT on these symptoms as well as the beneficial effects on bone and plasma cholesterol levels, its use has been limited because of side effects such as vaginal bleeding, breast tenderness, concerns about increased risks of breast cancer, and more recently, the concerns about cardiovascular safety in about 25% of patients. Given the clinical evidence that HT has an unfavorable benefit/risk ratio, there is an intense need for an alternative therapy for hot flushes and vaginal dryness. Both tamoxifen (TAM) and raloxifene (RAL) have shown their high therapeutic potential for estrogen-related diseases. Thus, TAM, the first SERM approved for breast cancer, is effective for all stages of hormone-dependent breast cancer, whereas RAL is indicated for the prevention and treatment of osteoporosis in postmenopausal women.[5] These SERMs have beneficial effects on bone and lipid metabolism, while antagonizing the effects of estrogens on the uterus and breasts. However, there are no SERMs reported to have a clinical beneficial effect on hot flushes and vaginal dryness.[4b,6]

Upon examining several recently reported potent SERM-pharmacophores, we noted in the tricyclic benzopyran scaffold of **7** (EM-652), the pendant phenolic ring D is orthogonal to the benzopyran ring (Figure 1). In contrast, in **8** (LY-357489), C and D rings were tied up into an almost planar tetracyclic backbone that resulted in what is reported as one of the most potent SERMs.[5c,d] We envisioned that SERMs with beneficial effects on hot flushes and potentially vaginal dryness (i.e. agonists on these tissues) should have a conformation in the C/D region similar to that of the natural ligand, 17β-estradiol. Therefore, structures with flexibility in the C/D region between **7** and **8** might offer distinct pharmacological profile. We designed such structures by replacing the C=C bond in the C ring of **8** with a CH_2O linker. We believe these bisbenzopyran analogues (**I**) closely mimic the backbone of steroidal estrogen estradiol (E_2) as well as **8** and perhaps could possess unique pharmacological properties.

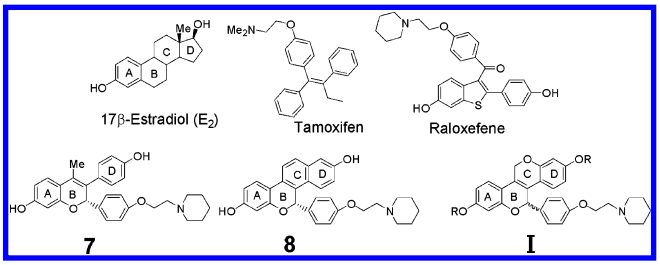


Figure 1. Structure of estradiol and SERMs.

The general synthetic approach of bisbenzopyran structure (**I**) is described in Scheme 1. The construction of the key tetracyclic chromene began with capping the phenolic hydroxyl groups of **3a** to form the *tert*-butyldimethylsilyl (TBDMS) derivative **3b** and then reduction with DIBAL-H to cleanly afford the lactol **4**.[7,8] Addition of a Grignard or lithium derivative of the dialkylaminoalkoxyphenyl side chain to lactol **4** opened the pyran ring B to afford the diol **5(a−f),** which recyclized into chromene structure **6(a−f)** under either Mitsunobu protocol or more conveniently by low-temperature acid treatment (concd HCl) in a hydrocarbon solvent such as toluene. Removal of the silyl groups from **6** with tetrabutylammonium fluoride (TBAF) afforded the target **2(a−f)** as racemic mixtures.

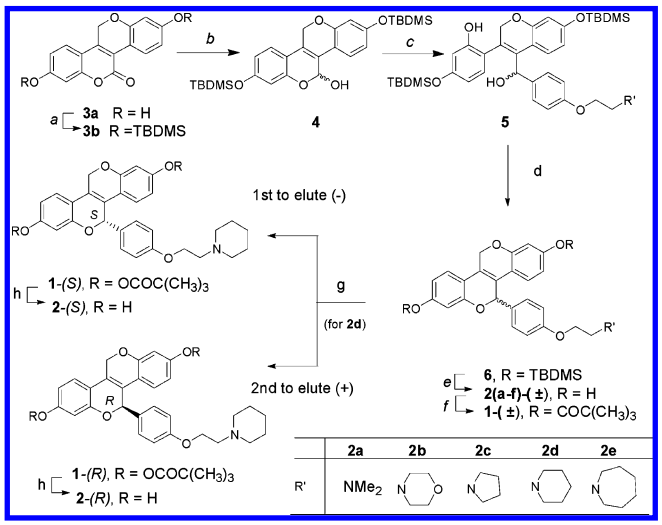
The choice of R′ group was significant in influencing affinity as well as potency in ER cell-based functional assays (Table 1). Compound **2a** (R′ = NMe_2) and **2c** (R′ = pyrrolidinyl) had similar binding affinity to ERs and exhibited antagonistic potency in endometrium Ishikawa cells as well human breast cancer cell line MCF-7. Compound **2b** with R′ as a morpholinyl group had good binding affinity, but weak inhibitory activity in MCF-7 cells. Piperidine was the best choice at this position as shown with the profile of compound **2d**. This compound had greater binding affinity in ER binding assays and also showed good potency in both Ishikawa as well as MCF-7 cell-based functional assays. When R′ was further functionalized as azepine (**2e**), both binding affinity and potency were significantly reduced. It was interesting to note that when OCH_2CH_2R' was absent, the compound (**2f**, structure not shown) showed agonistic activity in MCF as well as Ishikawa cells. Introduction of a *p*-*N*,*N*-dimethyl group to the side chain phenyl ring of **2f** (**2g,** structure not shown) converted the agonistic activity into antagonistic activity in Ishikawa cells while maintaining agonistic activity in MCF 7 cells. Raloxifene was used as a standard throughout the studies. In in vitro studies, our lead compound, **2d-(±)**, was slightly more potent.

To evaluate in vitro as well as in vivo pharmacology of **2d**-(*R*) and **2d**-(*S*), several attempts were made for chiral separation of racemic **2d**. During these efforts we realized free phenolic groups in the bisbenzopyrans **I** appeared to be sensitive to the

[a] SERM, selective estrogen receptor modulators; HT, hormone therapy; RAL, raloxifene; TAM, tamoxifen; ovex, ovariectomized.

10.1021/jm060353u CCC: $33.50
Published on Web 05/11/2006

Scheme 1[a]



[a] Reagents and conditions: a, TBSCl/Et_3N/DCM/ rt; b, DIBAL-H/ toluene/DCM/−78 °C; c, M-Ar, where M = MgI or Li; d, diethyl azidodicarboxylate, DEAD/THF/PPh_3 /rt or concd HCl/toluene/0−10 °C; e, TBAF/THF/ rt;f, pivaloyl chloride/Et_3N/DCM/rt. g, ChiralPak A/(5 cm × 50 cm, 20 m), 20% MeOH in IPA as mobile phase; h, Et_2NH (excess), MeOH, 60 °C in sealed tub.

Table 1. SAR of Chromene-Derived Bisbenzopyrans[a]

compd	ERα binding RBA, %	ERβ binding RBA, %	Ishikawa cells,[b] IC_{50} (nM)	MCF-7 cells,[c] IC_{50} (nM)
2a	61	40	180	220
2b	31	90	410	1400
2c	35	47	80	150
2d	340	170	12	110
2e	55	50	13	660
2f	13	110	(24)[d]	(94)[d]
2g	6.4	42	3800	(71)[d]
raloxifene	64	58	23	222

[a] All compounds were tested more than three times in these assays with high reproducibility. The average is shown here. [b] No agonist activity were found at 10 μM concentration except compound **2f**. [c] No agonist activity were found at 10 μM concentration except compound **2f** and **2g**. [d] Agonists, EC_{50} shown in parentheses; showed 13−22% inhibition at 10 μM concentration in antagonist format.

ambient conditions. It slowly decomposed upon long standing. Decomposition was also observed during the chiral separations using commercial available preparative chiral columns such as chiralPak-AD or chiralPak-OD. We, therefore, decided to use a pro-drug approach, where both phenolic groups were protected as the pivaloyl esters. The pivaloylation of **2d** was carried out using pivaloyl chloride and Et_3N and resulted in a white stable solid **1**-(±). Chromene **1**-(±) was resolved using preparative chiral HPLC (Chiralpak-AD column and IPA as mobile phase) to yield the title compounds **1**-(*S*) which was first to elute and **1**-(*R*) which was second to elute during chiral separation.[9a] Heating **1**-(*R*) and **1**-(*S*) in sealed tube at 55 °C with 1:1 mixture of diethylamine and MeOH afforded **2d**-(*R*) and **2d**-(*S*), respectively.[9b]

Both **1**-(*R*) and **1**-(*S*) were evaluated in in vitro assays for their estrogenic as well antiestrogenic activities and were compared with raloxifene and ethinyl estradiol.[10] In binding assays, neither **1**-(*R*) nor **1**-(*S*) binds to the purified estrogen receptors ERα or ERβ. The active metabolites **2d**-(*R*) and **2d**-(*S*) of **1**-(*R*) and **1**-(*S*) bind to both ERα and ERβ with affinities similar to raloxifene and ethinyl estradiol. Both **1**-(*R*) and **1**-(*S*) demonstrated antiestrogen activity in the two cell-based assays, MCF-7 breast cancer cell proliferation assay and Ishikawa endometrial cell alkaline phosphatase assay.[11] The active metabolites **2**-(*R*) and **2**-(*S*) showed activity in both functional assays, with similar potency to raloxifene. These compounds, **1**-(*R*) and **1**-(*S*) or **2d**-(*R*) and **2d**-(*S*), were devoid of any stimulatory activity in human MCF-7 breast cancer cells or Ishikawa endometrial cell assay, indicative of antiestrogenic activity in these cell lines. Table 2 summarizes the in vitro profiles for **1**-(*R*), **1**-(*S*), **2d**-(*R*), and **2d**-(*S*).

Table 2. In Vitro Profiles of 1-(*R*) and 1-(*S*) and Its Active Metabolites **2d**-(*R*) and **2d**-(*S*)[a]

compd	ERα binding IC_{50} (nM)	ERβ binding IC_{50} (nM)	human endometrial Ishikawa cells[b] IC_{50} (nM)	human breast MCF-7 cells[b] IC_{50} (nM)
ethinyl estradiol	2.0	8.1	NA	NA
raloxifene	1.8	8.2	24.6	222
1-(*R*)	>10000	>10000	175[c]	510[c]
2d-(*R*)	51	20.3	43.3	599
1-(*S*)	>1000	>10000	99[c]	670[c]
2d-(*S*)	0.61	1.3	17.1	416

[a] All compounds were tested more than three times in these assays with high reproducibility. The average is shown here. [b] No agonist activity was found at the 10 μM concentration. [c] **1**-(*R*) and **1**-(*S*) may be getting hydrolyzed to **2d**-(*R*) and **2d**-(*S*), respectively, in these cell-based functional assay.[17]

Both **1**-(*R*) and **1**-(*S*) were evaluated in several in vivo models for estrogen action. These models were used to assess the tissue selective activity of the compound. In these studies, **1**-(*R*) and **1**-*S)* were orally administered to animals in an aqueous homogeneous suspension (in 0.5% Methocel) daily in all studies except the hot flush assay. In the latter, sesame oil was used as the vehicle. The active drug components **2**-(*R*) and **2**-(*S*) were not evaluated for the in vivo activity because of their long-term stability concerns.[12]

Compounds **1**-(*R*) and **1**-(*S)* were tested for their in vivo effects on uterine weight in immature rats (uterotopic assay). This assay is used to demonstrate in vivo activity.[18a] In the agonist mode, **1**-(*R*) and **1**-(*S*) were administered orally once daily (1.4 mg/kg) for 3 days and the effect on uterine wet weight was compared with vehicle, estrone, tamoxifen, and raloxifene.[18b] In these uterotopic assays, when **1**-(*R*), **1**-(*S*), or raloxifene were administered alone, they showed no significant agonistic activity to stimulate uterine growth, while estrone and tamoxifen significantly increased the uterine wet weight. In the antagonist mode, the daily oral administration (3 days) of **1**-(*R*) and **1**-(*S*) were studied to evaluate the ability of the compounds to block the increase in uterine wet weight induced by estrone. Oral administration (3 days) of both **1**-(*R*) and **1**-(*S*) led to a decrease in uterine wet weight in a dose dependent manner to 27%, 55%, and 75% inhibition of estrone-stimulated uterine weight increase at 0.14, 0.42, and 1.4 mg/kg doses, respectively while 1-(*S*) led to 55%, 88%, and 100% inhibition of estrone-stimulated uterine weight increase at respective doses of 0.14, 0.42, and 1.4 mg/kg. The effects of **1**-(*R*) and **1**-(*S*) on estrone-induced uterine weight in immature rats were similar to raloxifene.[20]

Next we examined the effects of **1**-(*R*) and **1**-(*S*) on adult ovariectomized (ovex) rats to see the estrogenic effects on multiple tissues, including uterus, vagina, bone, and liver (plasma lipids). For these studies, the rats were ovariectomized and then treated, orally, with the compounds **1**-(*R*), **1**-(*S*), and raloxifene for 6 weeks. Neither **1**-(*R*) nor **1**-(*S*) significantly increased uterine wet weight when administered alone to the ovex rats. Similar to the data on uterine weight, **1**-(*R*) and **1**-(*S*) had minimal effect on epithelial cell height, similar to raloxifene.[14] To assess the skeletal effect of **1**-(*R*) and **1**-(*S*) in these animals, total and trabecular bone mineral densities of the tibia

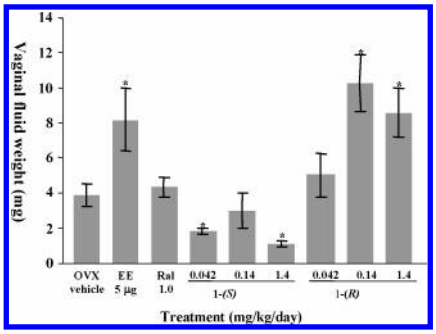


Figure 2. Effect of 2 weeks of treatment with **1**-(*R*) on vaginal fluidity levels in comparison to ethynylestradiol, raloxifene, and **1**-(*S*) in ovariectomized rats. *Significantly different from OVX control ($p < 0.05$).

were measured with pQCT. Both **1**-(*R*) and **1**-(*S*) effectively prevented bone loss in a dose-dependent manner, and to a similar level as raloxifene.[20] Following 6 weeks of treatment, blood was collected and total cholesterol levels were determined in the serum. **1**-(*R*) and **1**-(*S*) both reduced the circulating serum total cholesterol levels at all doses tested. The maximum reduction was to approximately 33−35% that of the control vehicle treated animals. These responses for bone density and cholesterol were similar to that seen in raloxifene-treated animals and reported previously.[14, 20]

Importantly, in these same ovex animals there was a key differentiation from raloxifene, that is, the impact on the vagina. Raloxifene has no beneficial effect on vaginal dryness, a critical cause for genital complaints in postmenopausal women.[3] We studied effect of **1**-(*R*), **1**-(*S*), and raloxefene on the production of vaginal fluid in ovex rats. The fluid in the vagina was collected using a cotton swab and the absolute weight of the fluid was measured for each animal after 2 weeks of treatment (Figure 2). In addition, the cytology of the vaginal smear from each animal was evaluated. Cytological observation showed that ovex rats treated with vehicle, **1**-(*S*), **1**-(*R*), and raloxifene were all in diestrus, while the rats treated with ethinyl estradiol were in estrus. Ethinyl estradiol showed an increase in vaginal fluid, while raloxifene showed no effect. Compound **1**-(*R*) increased the vaginal fluid in ovex rats at the 0.14 mg/kg/day and 1.4 mg/kg/day doses but not at lower doses and which are comparable to ethinyl estradiol, while the enantiomer **1**-(*S*) decreased the vaginal fluid in ovex rats.

On the basis of literature evidence, a change in tail temperature of morphine dependent rats, following morphine withdrawal, reflects the human symptoms of a hot flush.[16b] This hot flush model has been used to characterize several SERMs.[5b,15,16] We also evaluated **1**-(*R*) and **1**-(*S*) in the hot flush model. Our data demonstrated that **1**-(*R*) effectively suppresses the increase of tail skin temperature $\Delta T = 1.51$ at 1.4 mg /kg.day while raloxifene had no effect in suppressing the tail skin temperature (Figure 3). In a separate experiment, the values of AUC (area under curve) from the first 15 min following morphine withdrawal were also measured (Figure 4). In a separate study (data not shown), **1**-(*S*) had no effect on suppressing the tail skin temperature. The result from this hot flush model indicates that **1**-(*R*) may have therapeutic efficacy for hot flushes.

It is interesting to note that both **1**-(*R*) and **1**-(*S*) have similar in vitro as well pharmacokinetic properties.[17] However **1**-(*R*) relieves hot flushes (agonist effect on thermoregulatory center) and increases vaginal fluidity (agonist effect on vagina) while **1**-(*S*) seems to elevate hot flushes (antagonist effect on thermoregulatory center) as well as increase vaginal dryness

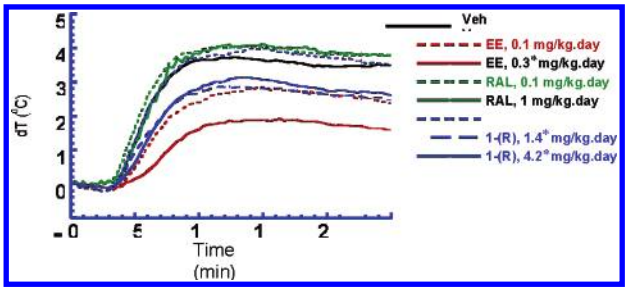


Figure 3. Effect of **1**-(*R*) on the change in tail skin temperature in a morphine-dependent rat model. ΔT represents the mean of maximum change in tail skin temperature relative to baseline; po, $n = 24$ vs vehicle group. *Significantly different from ovex control ($p < 0.05$).

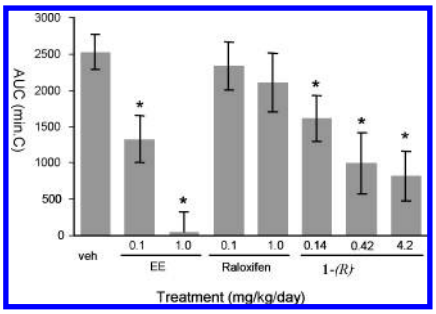


Figure 4. Effect of **1**-(*R*) in rat hot flush model illustrated by AUC. *Significantly different from vehicle control ($p < 0.05$, $n = 24$).

(antagonist on vagina). Such drastic changes in pharmacology could be attributed to certain changes in conformation of the ligand-bound receptor complex and its interaction with the coactivators and corepressors.[19]

In conclusion, we discovered a series of nonsteroidal estrogen receptor modulators with chromene-derived bisbenzopyran as the core structure. Our data from in vivo studies demonstrated that **1**-(*R*) possesses ideal SERM profile in animal studies. It not only exhibits estrogen agonistic effects on bone and lipid, and antagonistic effects on mammary glands and uterus, but also alleviates hot flushes and increases the amount of vaginal fluid. This unique pharmacological profile made compound **1**-(*R*) an ideal clinical candidate for treatment of hot flushes and vaginal dryness in postmenopausal women, without the risk of uterine or breast cell stimulation. The biology and pharmacology associated with the unique profile of **1**-(*R*) as well as results from detailed preclinical and clinical studies will be reported in due course.

Acknowledgment. We are grateful to Drs. Rehka Shah, Yong Zhou, and Brigitte Segmuller for stereochemistry discussions and Dr. Mark J. Macielag for his guidance.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent reviews, see the following: (a) Jordan, V. C. Antiestrogens and Selective Estrogen Receptor Modulators as Multifunctional Medicines., Receptor Interactions. *J. Med. Chem.* **2003**, 46, 883−908. (b) Jordan, V. C. Antiestrogens and Selective Estrogen Receptor Modulators as Multifunctional Medicines, Clinical Considerations and New Agents. *J. Med. Chem.* **2003**, *46*, 1081−1111. (c) Riggs, B. L.; Hartmann, L. C. Selective Estrogen-Receptor Modulators-Mechanisms of Action and Application to Clinical Practice. N. *Engl. J. Med.* **2003**, *348*, 618−629. (d) Freedman, R. R. Physiology of Hot Flashes. *Am. J. Hum. Biol.* **2001**, *13*, 454−464.

(2) (a) Shanafelt, T. D.; Barton, D. L.; Adjei, A. A.; Loprinzi, C. L. Pathphysiology and Treatment of Hot Flashes. *Mayo Clin. Proc.* **2002**, *77*, 1207−1218.

(3) For recent reports, see the following: Simunic, V.; Banovic, I.; Ciglar, S.; Jeren, L.; Pavicic Baldani, D.; Sprem, M. Local estrogen treatment in patients with urogenital symptoms. *Intl. J. Gynecol. Obstet.* **2003**, *82* (2), 187−197 (b) Miller, C. P. SERMs: Evolutionary Chemistry, Revolutionary Biology. *Curr. Pharm. Des.* **2002**, *8* (23), 2089−2111 (c) Kenemans, P.; Speroff, L. SERMs: Evolutionary Chemistry, Revolutionary Biology. Maturitas **2005**, *51* (1), 21−28 (d) Oskay, U. Y.; Beji, N. K.; Yalcin, O. Acta obstetrician A study on urogenital complaints of postmenopausal women aged 50 and over. *Gynecol. Scand.* **2005**, *84* (1), 72−78. (e) Bachmann G. Urogenital aging: an old problem newly recognized. *Maturitas* **1995**, 22 Suppl S1−S5.

(4) (a) Seibel, M. M. Freedman, R. R. Physiology of Hot Flashes. *Am. J. Hum. Biol.* **2001**, *13*, 454−464. (b) Plouffe, L., Jr.; Facoq, C. M. Selective Estrogen Receptor Modulators (SERMs) in Clinical Practice. *J. Soc. Gynecol. Invest*. **2000**, *7*, S38−S46.

(5) (a) Hulley, S.; Grady, D.; Bush, T.; et al. Randomized Trial of Estrogen plus Progestin for Secondary Prevention of Coronary Heart Disease in Postmenopausal Women. *J. Am. Med. Assoc.* **1998**, *280*, 605−613. (b) Collaborative Group on Hormonal Factors in Breast Cancer. Breast Cancer and Hormone Replacement Therapy. *Lancet* **1997**, *350*, 1047−1059 (c) Gauthier, Sylvain; Cloutier, Julie; Dory, Yves L.; Favre, Alexandre; Mailhot, Josee; Ouellet, Carl; Schwerdtfeger, Annette; Merand, Yves; Martel, Celine; Simard, Jacques; Labrie, Fernand. Synthesis and structure−activity relationships of analogues of EM-652 (acolbifene), a pure selective estrogen receptor modulator. *J. Enzyme Inhib. Med. Chem.* **2005**, *20* (2), 165−177. (d) Grese, T. A.; Pennington, L. D.; Sluka, J. P.; Adrian, M. D.; Cole, H. W.; Fuson, T. R.; Magee, D. E.; Phillips, D. L.; Rowley, E. R.; Shetler, P. K.; Short, L. L.; Venugopalan, M.; Yang, N. N.; Sato, M.; Glasebrook, A. L.; Bryant, H. U. Synthesis and pharmacology of conformationally restricted raloxifene analogues: highly potent selective estrogen receptor modulators. *J. Med. Chem.* **1998**, *41*, 1272.

(6) (a) Davies, G. C.; Huster, M. W. J.; Lu, Y.; Plouffe, L., Jr.; Lakshmanan, M. Adverse Events Reported by Postmenopausal Women in Controlled Trials with Raloxifene. *Obstet. Gynecol.* **1999**, *93*, 558−565. (b) Loprinzi, C. L.; Zahasky, K. M.; Slaoan, J. A.; Novotny, P. J.; Quella, S. K. Tamoxifen-Induced Hot Flashes. *Clin. Breast Cancer* **2000**, *1*, 52−56.

(7) Kanojia, R. M.; Jain, N. F.; Ng, R.; Sui, Z.; Xu, J. Preparation of tetracyclic heterocycles as selective estrogen receptor modulators (SERMs) WO 2003053977, 2003. US 71997520031121, 2004.

(8) For synthesis of the tetracyclic core structure, see (a) Kanojia, R. M.; Jain, N.; Xu, J.; Sui, Z. Preparation of tetracyclic heterocycles as selective estrogen receptor modulators (SERMs). *Tetrahedron Lett.* **2004**, *45*, 5837−5839. (b) Jain, N.; Xu, J.; Chen, N.; Sui, Z. A novel approach to the synthesis of 11,11-dimethyl-bisbenzopyran-5-ones. *Tetrahedron Lett.* **2006**, *47*, 225−228.

(9) (a) The absolute configuration of **1**-(*R*) and **1**-(*S*) has been determined by VCD spectroscopy data. (b) ee values were > 95; optical purities were determined by chiral Pak AD column.

(10) Allan, G. F.; Hutchins, A.; Clancy, J. An ultrahigh-throughput screening assay for estrogen receptor ligands. *Anal. Biochem.* **1999**, *275*, 243−247. (b) Rufbin, V. N.; Ruenitz, P. C.; Boyd, J. L.; Boudinot, F. D.; Wiese, T. E. Characterization of Selective Estrogen Receptor Modulator (SERM) Activity in Two Triarylethylene Oxybutyric Acids. *Biochem. Pharmacol.* **2002**, *63*, 1517−1525.

(11) Wang, T. T.; Sathyamoorthy, N.; Phang, J. M. Molecular Effects of Genistein on Estrogen Receptor Mediated Pathways. *Carcinogenesis* **1996**, *17*, 271−275.

(12) Possible path way for auto oxidation is described in ref 8b.

(13) (a) Komm B. S.; Rusling D. J.; Lyttle C. R. Estrogen regulation of protein synthesis in the immature rat uterus: the analysis of proteins released into the medium during in vitro incubation. *Endocrinology* **1986**, *118*, 2411−2416. (b) Komm, B. S.; Kharode, Y. P.; Bodine, P. V. N.; Harris, H. A.; Miller, C. P.; Lyttle, C. R. Bazedoxifene acetate: a selective estrogen receptor modulator with improved selectivity. *Endocrinology* **2005**, *146* (9), 3999−4008.

(14) (a) Komm, B. S.; Lyttle, C. R. Developing a SERM: Stringent preclinical selection criteria leading to an acceptable candidate (WAY-140424) for clinical evaluation. *Ann.* N.Y. Acad. Sci. **2001**, *949*, 317−326. (b) Ashby J.; Odum, J.; Foster, J. R. Activity of raloxifene in immature and ovariectomized rat uterotrophic assays. *Toxicol Pharmacol* **1997**, *25*, 226−231. (c) Qu, Q.; Zheng, H.; Dahllund, J.; Laine, A.; Cockcroft, N.; Peng, Z.; Koskinen, M.; Hemminki, K.; Kangas, L.; Väänänen, K.; Härkönen, P. Selective estrogenic effects of a novel triphenylethylene compound, FC1271a, on bone, cholesterol level, and reproductive tissues in intact and ovariectomized rats. *Endocrinology* **2000**, *141*, 809−820. (d) Sato, M.; Turner, C. H.; Wang, T.; Adrian, M. D.; Rowley, E.; Bryant, H. U. J. LY353381.HCl: a novel raloxifene analogue with improved SERM potency and efficacy in vivo. *Pharmacol. Exp. Ther.* **1998**, *287*, 1−7.

(15) Geiser, A. G.; Hummel, C. W.; Draper, M. W.; Henck, J. W.; Cohen, I. R.; Rudmann, D. G.; Donnelly, K. B.; Adrian, M. D.; Shepherd, T. A.; Wallace, O. B.; McCann, D. J.; Oldham, S. W.; Bryant, H. U.; Sato, M.; Dodge, J. A. A new selective estrogen receptor modulator with potent uterine antagonist activity, agonist activity in bone, and minimal ovarian stimulation. *Endocrinology* **2005**, *146* (10), 4524−4535.

(16) (a) Simpkins, J. W.; Katovich, M. J.; Song, I.-C. Chronic hyperprolactinemia reduces peripheral beta-adrenergic responsiveness in male rats. *Life Sci.* **1983**, *32*, 1957−1966. (b) Katovich, M. J.; Simpkins, J. W.; Berglund, L. A.; O'Meara, J. Regional skin temperature changes in a rat model for the menopausal hot flush. *Maturitas* **1986**, *8*, 67−76. (c) Merchenthaler, I.; Funkhouser, J. M.; Carver, J. M.; Lundeen, S. G.; Ghosh, K.; Winneker, R. C. The effect of estrogens and antiestrogens in a rat model for hot flush. *Maturitas* **1998**, *30*, 307−316.

(17) Both **1**-(*R*) and **1**-(*S*) have similar pharmacokinetic properties in rats. The active metabolites **2d**-(*R*) and **2d**-(*S*) were detected in plasma predominantly with similar exposures as compared to the parent compounds **1**-(*R*) and **1**-(*S*), respectively. The total bioavailability calculated for **1**-(*R*) and **1**-(*S*) was about 11% and 9%, respectively. The partial hydrolyzed products were not detected in rat plasma.

(18) (a) The concentration of these compounds in blood was not determined in the immature uterotropic assays. (b) From our PK study, we have shown that **1**-(*R*) and **1**-(*S*) are extensively metabolized to **2d**-(*R*) and **2d**-(*S*), respectively. To correct the molecular weight change, a 1.4 mg/kg dose was chosen as standard screening dose.

(19) (a) Matsumoto, T.; Morii, H.; Yazaki, Y.; Ouchi, Y.. Eds. Selective Estrogen Receptor Modulators. *Clin. Calcium* **2002**, *12* (12), 102 pp. (b) For a detailed description of the current understanding of tissue-specific steroid hormone activities, see Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. Tripartite steroid hormone receptor pharmacology: Interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Mol. Endocrinol.* **1996**, *10*, 119−131.

(20) See Supporting Information for details.

JM060353U