Discovery and Preclinical Characterization of (+)-3-[4-(1-Piperidinoethoxy)phenyl]spiro[indene-1,1′-indane]-5,5′-diol Hydrochloride: A Promising Nonsteroidal Estrogen Receptor Agonist for Hot Flush

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Abstract: In our studies of the development of a novel class of selective estrogen receptor modulators, (+)-3-[4-(1-piperidinoethoxy)phenyl]spiro[indene-1,1′-indane]-5,5′-diol hydrochloride (**1**) was found to be an estrogen receptor ligand with beneficial effects in rat models for human hot flush. Moreover, **1** was found to have beneficial effects on lipid and bone metabolism while maintaining marginal effects on the uterus and breasts. These findings suggest that **1** would provide a new treatment for hot flush.

Introduction. Hot flush, the most common symptom of the climacteric, is characterized by the sudden onset of hot feeling, sweating, palpitation, and anxiety and affects approximately 75% of naturally menopausal women. The symptom begins 1−2 years before menopause and lasts for 6 months to 5 years. In women with surgical or chemotherapy-induced menopause, the symptom generally lasts longer and is more frequent and severe than that associated with natural menopause.[1] Although the physiological mechanism for hot flush remains unclear, a decline in circulating estrogen levels has been thought to cause dysfunction of the central thermoregulatory centers.[2a]

Indeed, hormone replacement therapy (HRT) alleviates the symptom in 80−90% of women and has been recognized as the most effective treatment for hot flush.[2b] Despite the effectiveness of HRT, its use has been limited because of side effects such as vaginal bleeding and breast tenderness and because of concerns about increased risks of hormone-dependent cancer.[3] Very recently, HRT was found to increase the risks of cardiovascular events in women with coronary heart disease and breast cancer when undergone for a period of 5 years or more.[4] Given clinical evidence that HRT has an unfavorable benefit/risk ratio, there is an intense need for an alternative therapy for hot flush. With the advent of tamoxifen (TAM) and raloxifene (RAL), selective estrogen receptor modulators (SERMs) have shown their high therapeutic potential for estrogen-related diseases.[5] Thus, TAM, the first SERM to be approved for the prevention of breast cancer in high-risk women, is effective for all stages of hormone-dependent breast cancer, whereas RAL is prescribed for the prevention and treatment of osteoporosis in postmenopausal women.[6] While SERMs on the market have beneficial effects on bone and lipid metabolism antagonizing the effects of estrogens on the uterus and breasts, no SERM has been reported to have a beneficial effect on hot flush.[6,7] In our studies of the development of a novel class of SERMs with beneficial effects on hot flush, we have found spiro[indene-1,1′-indane]-5,5′-diol as a new framework for estrogen receptor ligands and assumed that a series of related compounds might be imparted with unprecedented biological properties due to their new structural characteristics.[8] Herein, we disclose the synthesis and unique biological profile of (+)-3-[4-(1-piperidinoethoxy)phenyl]spiro[indene-1,1′-indane]-5,5′-diol hydrochloride [**1**, OS-689; *ent*-**1**, **2**; Figure 1].[9]

Chemistry. We envisaged that 1,1′-spirobiindan-3-one (**8**), a key intermediate, could be constructed by means of a rhodium(II) carboxylate catalyzed intramolecular carbon−hydrogen insertion reaction of α-diazo *β*-keto esters.[10] As shown in Scheme 1, addition of Grignard reagent **3**[11] to 5-methoxyindan-1-one (**4**) followed by acid-catalyzed dehydration[12] and hydrogenolysis with $Pd(OH)_2$−C gave the benzoic acid **5** in 62% yield. Conversion of **5** into the corresponding acid chloride followed by condensation with potassium enolate of ethyl acetate and subsequent diazo transfer reaction gave the α-diazo *β*-keto ester **6** in 96% yield. $Rh_2(OAc)_4$-catalyzed intramolecular carbon−hydrogen insertion reaction of **6** successfully proceeded in $PhCF_3$ at 60 °C to afford **7** as a single product in 82% yield. Deethoxycarbonylation of **7** gave **8** in quantitative yield. The basic side chain was incorporated by addition of the aryllithium reagent **9**, and subsequent dehydration with TsOH followed by demethylation[13] with Ph_2PLi gave a racemic mixture of **1** and **2** in 42% yield in three steps. At this stage, we investigated the possibility of optical resolution of **4** or **1** and **2** with a variety of resolving agents. While all resolution attempts failed, we instead found that the intermediate **10** could be separated by preparative chiral HPLC (Scheme 2). The basic side chain was assembled by removal of the allyl group in (+)-**10** with 5 mol % $Pd(OAc)_2$ in HCO_2H followed by Mitsunobu reaction with piperidineethanol. Subsequent demethylation gave **1** with up to 99% ee.

Results and Discussion. The binding affinity for estrogen receptor (ER) was determined by a competitive radiometric binding assay in human breast cancer (MCF-7) cytosol, using [^{3}H]-17*β*-estradiol as tracer.[14] While both **1** and **2** showed good affinity for the ER, **1** had higher affinity (K_i = 9.05 nM) than **2** (K_i = 78.3 nM).[15] Somewhat surprisingly, as will be discussed below, **1** was more potent in terms of in vivo biological activity than was predicted from its binding affinity. Because it is known that two subtypes of the estrogen receptor, ERα and ER*β*, may have different biological roles[16] and that ERα is expressed primarily in the breast and uterus (only in MCF-7 cells) whereas ER*β* is found mainly in the brain and bone, ER*β* may be involved in

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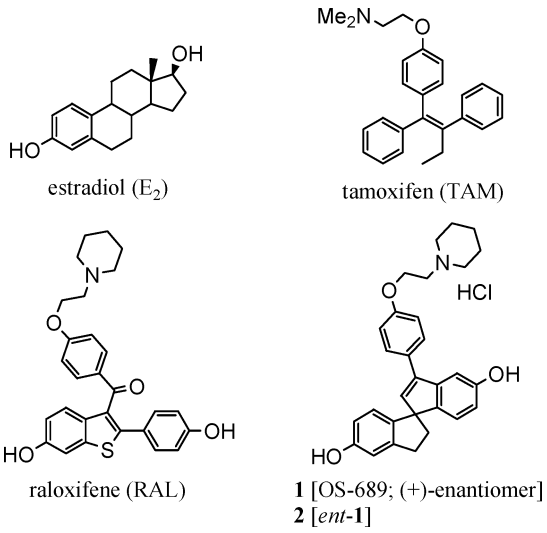
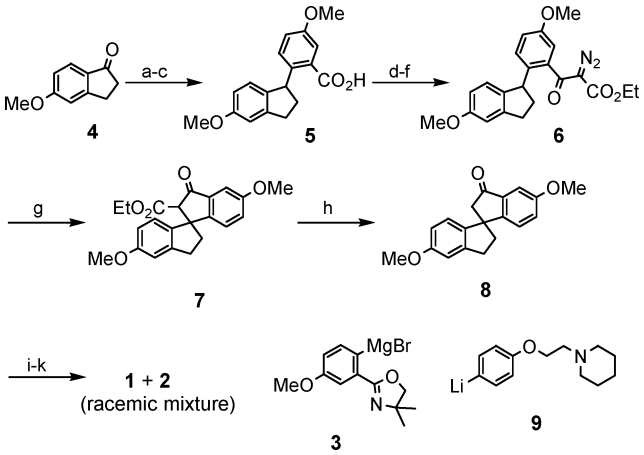


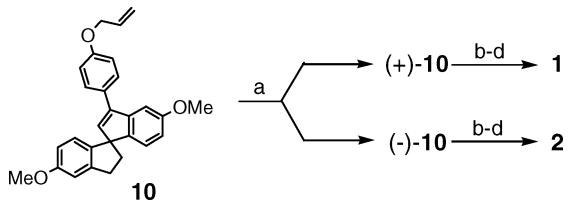
Figure 1. Structures of estradiol and SERMs.

Scheme 1[a]



[a] Reagents and conditions: (a) **3**, THF; (b) 6 N H_2SO_4, dioxane, reflux; (c) H_2 (1 atm), 20% $Pd(OH)_2$−C, AcOH; (d) $(COCl)_2$, toluene; (e) AcOEt, KHMDS, THF; (f) *p*-acetoamidobenzenesulfonyl azide, Et_3N, MeCN; (g) 0.5 mol % $Rh_2(OAc)_4$, $PhCF_3$, 60 °C; (h) 90% aqueous DMSO, 150 °C; (i) **9**, THF, −78 °C; (j) TsOH, toluene, reflux; (k) Ph_2PH, *n*-BuLi, THF, reflux.

Scheme 2[a]



[a] Reagents and conditions: (a) HPLC chiral separation with a CHIRALPAK OJ column; (b) HCO_2H, $Pd(OAc)_2$, Ph_3P, benzene, reflux; (c) piperidineethanol, DIAZO, Ph_3P, THF; (d) Ph_2PH, *n*-BuLi, THF, reflux.

this discrepancy.[17,18] Besides the possibility of the involvement of ERβ, it has been reported that the binding affinity does not necessarily correlate with the conformation of the receptor−ligand complex, which affects the transcriptional or nongenotropic activity, while the binding affinity is a dominant factor for a ligand potency.[19] Additionally, in the case of oral administration, pharmacokinetic factors such as absorption, distribution, and metabolism must be taken into account.

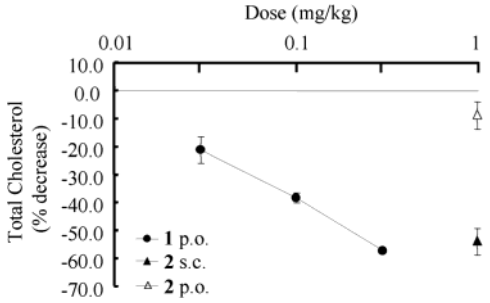


Figure 2. Effects of **1** and **2** on plasma cholesterol in male rats. Sprague−Dawley (SD) rats (6- to 8-week old) were treated daily for 4 days with vehicle control (solid line), **1** (closed circles), or **2** (oral administration, open triangle; subcutaneous administration, closed triangle) at the indicated doses. Each point represents the mean percent decrease (±SEM) relative to vehicle with a group size of $n = 5$. The observed effects of **1** at all doses differ significantly from those of the vehicle control at $P < 0.05$.

Further explanation for the discrepancy should be limited.[20]

At the outset of our in vivo investigation, the cholesterol-lowering effects of **1** and **2** were briefly compared. As can been seen from Figure 2, orally administered **1** dose-dependently decreased serum cholesterol in rats with significant effect at a dose of 0.03 mg/(kg·day).[21] However, **2**, given orally even at a dose of 1 mg/(kg·day), had no effect on rats cholesterol level. The inefficacy of orally administered **2** seems to be partly due to its poor oral bioavailability because subcutaneous administration of **2** produced a decrease in rats cholesterol levels similar to that of **1**. Hence, we used **1** for further evaluation.

On the basis of accumulating evidence that the change in tail temperature of ovariectomized (OVX) rats reflects human symptoms of skin temperature fluctuation, we chose the OVX rat model as a principal model for human hot flush.[22] As has previously been reported, tail skin temperature of OVX rats was significantly higher than that of sham-operated controls, and estradiol (E_2) attenuated this ovariectomy-induced rise in tail skin temperature (Figure 3A). Likewise, **1** dose-dependently attenuated the rise in tail temperature of OVX rats and completely restored tail temperature to the level of sham-operated controls at doses as low as 0.1 mg/(kg·day). In addition, **1** did not antagonize the effect of E_2 on the change in tail temperature at any dose (0.1−10 mg/(kg·day), Figure 3B). These results suggest that **1** acted as a full estrogen agonist in the thermoregulatory centers. This suggestion is supported by results from the morphine-dependent rat model.[23] Thus, Merchenthaler[23c] has reported that E_2 suppresses the rise in tail temperature of morphine-dependent OVX rats induced by naloxone injection and that RAL has no effect on the change in tail temperature. In our experiment with a slightly modified method, although E_2 and RAL gave similar results as those mentioned above, **1** suppressed the rise in tail temperature with potency virtually similar to that of E_2 (Figure 4). All results from the two models indicate that **1** may have a therapeutic efficacy on hot flush.

Next, we examined the preventive effects of **1** on osteoporosis.[24] **1** prevented the loss of total bone mineral density of the distal femur with a significant effect at a

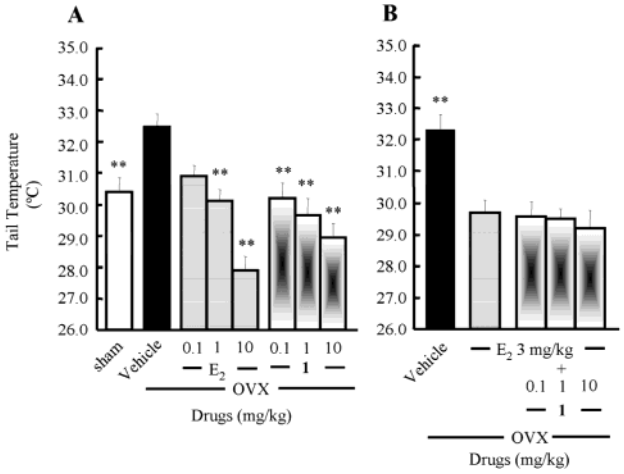


Figure 3. (A) Effects of E_2 and **1** on tail skin temperature of OVX rats. (B) Effects of **1** on the E_2-induced change in tail skin temperature of OVX rats. Two weeks after ovariectomy, OVX (SD) rats were orally treated daily for 10 days with E_2 alone, **1** alone, or **1** plus E_2 at the indicated doses. The OVX rats were allowed free movement as described,[22c] and tail temperature was measured for 90 min with a thermistor probe (Technol Seven, Japan) placed on the tail approximately 5 cm from its base. Data were analyzed by the Fluclet software system (Dainippon Pharmaceutical Co., Japan), and values represent the mean tail skin temperature (±SEM) relative to vehicle with a group size of $n = 7$. (**) $P < 0.01$: vs vehicle group (A). (**) $P < 0.01$: vs E_2 alone group (B).

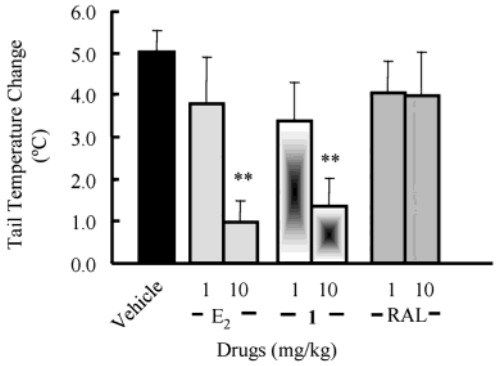


Figure 4. Effects of **1** on the change in tail skin temperature in a morphine-dependent rat model. Ten days after ovariectomy, OVX (SD) rats were orally treated daily at 5:00 p.m. for 8 days with E_2, **1**, or RAL at the indicated doses. From the third day to the seventh day of treatment, Kadian (Dainippon Pharmaceutical Co.), a slow releasing pellet of morphine was orally administered to the rats at 9:00 a.m. and its dose was increased by a staircase method from 20 mg/(kg·day) (morphine base) to 60 mg/(kg·day). On the eighth day of treatment, measurement of tail temperature was carried out as described in Figure 3 under ketamine (80 mg/kg, im) anesthesia. The baseline was recorded for 15 min, and tail skin temperature measurement was continued for at most 1 h after naloxone-induced (1 mg/kg, sc) morphine withdrawal. Values represent the mean of a maximum reponse of change in tail skin temperature (±SEM) relative to baseline with a group size of $n = 7-10$. (**) $P < 0.01$: vs vehicle group.

dose of 0.1 mg/(kg·day) and an efficacy as potent as that of E_2 or RAL (Figure 5).

These encouraging results led us to investigate critical issues related to adverse effects. In OVX rat model, **1** had minimal stimulatory activity toward the uterus (Figure 6A).[24a] On the other hand, in an immature rat model, **1** at doses of 0.1−10 mg/kg acted as a weak estrogen agonist in the uterus (Figure 6B), showing slightly

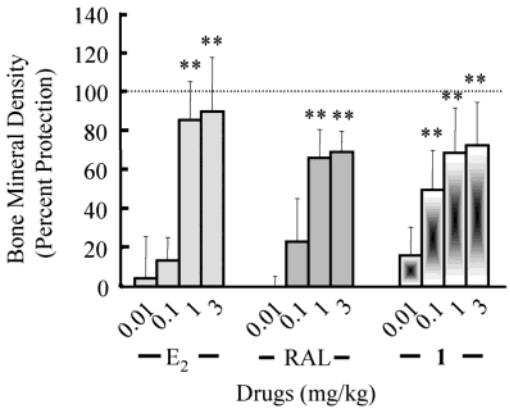


Figure 5. Effects of E_2, RAL, and **1** on bone mineral density (BMD) in OVX rats. SD rats (9-week old) were ovariectomized and orally treated daily for 4 weeks with E_2, RAL, or **1** at the indicated doses. Values represent the mean percent protection (±SEM) relative to OVX controls, with sham control values defined as 100% (dotted line) and OVX control defined as 0%. The group size was $n = 8$. (**) $P < 0.01$: vs vehicle group.

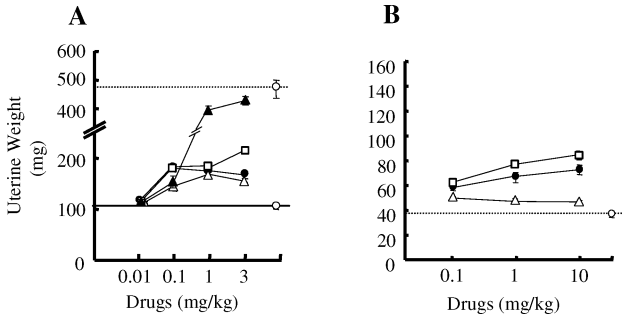


Figure 6. (A) Comparison of the effects of **1**, E_2, RAL, and TAM on uterine wet weight in OVX rats. SD rats (9-week old) were ovariectomized and orally treated daily for 4 weeks with **1** (closed circles), E_2 (closed triangles), RAL (open triangles), and TAM (open squares) at the indicated doses. Uteri were removed and weighed. Each point represents the mean (±SEM) with a group size of $n = 8-16$. Two sets of controls are shown: vehicle (solid line) and sham (dotted line). The observed effects of all test compounds at doses of more than 0.1 mg/(kg·day) differ significantly from those of OVX control at $P < 0.01$. (B) Comparison of the effects of **1**, RAL, and TAM on uterine wet weight in immature rats. SD rats (21-day old) were orally treated daily for 4 days with **1** (closed circles), RAL (open triangles), and TAM (open squares) at the indicated doses. Uteri were removed and weighed. Each point represents the mean (±SEM) with a group size of $n = 5$, and the dotted line indicates sham controls. The observed effects of test compounds in all experiments differ significantly from those of the vehicle control at $P < 0.01$.

higher stimulatory activity than that of RAL.[24a] Finally, in the MCF-7 proliferation assay,[14a,25] **1** showed considerably weak stimulatory and inhibitory effects on cell proliferation.[26] Taken together, it seems that the intrinsic estrogenic effects of **1** on the reproductive tissues are only very modest and much less than those of E_2.

In conclusion, we synthesized **1**, a nonsteroidal estrogen receptor ligand with a spiro[indene-1,1′-indane] core structure, capitalizing on the rhodium(II) carboxylate catalyzed carbon−hydrogen insertion reaction. Our data from rat models show that **1** has beneficial effects on central thermoregulatory centers and bone and lipid metabolism while maintaining marginal effects on the uterus and breasts. These findings suggest that **1** would meet the criteria as a SERM and provide an alternative

to HRT. Although the biological mechanism by which **1** exerts its unique pharmacological effects is an open question, our results would facilitate the development of a new generation SERMs.

Supporting Information Available: Experimental procedures for the synthesis of **1**, and physical and spectral data for key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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