Letters

Design, Synthesis, and Preclinical Characterization of Novel, Highly Selective Indole Estrogens

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Received February 27, 2001

Introduction. The onset of menopause has been associated with a reduction in endogenous estrogen levels that has been correlated with a variety of menopausal symptoms and an increased risk for osteoporosis and heart disease.[1] Hormone replacement therapy successfully alleviates these symptoms, but concerns about hyperplastic estrogen action on breast and uterine tissue have been raised.[2,3] While the hyperplastic action of estrogen on uterine tissue can be successfully opposed by the coadministration of a progestin, the effects of hormone replacement therapy on breast tissue remain controversial and is the subject of some debate.[4] Largely as a result of these issues, considerable research has been dedicated to the discovery of estrogens that act in a tissue selective fashion.[5] The prototypical estrogen with some tissue selectivity is, paradoxically, the "anti-estrogen", tamoxifen **1** (Figure 1). While tamoxifen has been used for over two decades in the treatment of hormone-dependent breast cancer, it was not until 1987 that Jordan and co-workers reported that tamoxifen was able to protect against bone loss in the ovariectomized rat model.[6] Jordan's group also demonstrated that raloxifene **2** (raloxifene hydrochloride is currently Evista for osteoporosis) was also effective as an estrogen agonist on bone in the ovariectomized rat, while displaying even less uterine stimulation than tamoxifen.

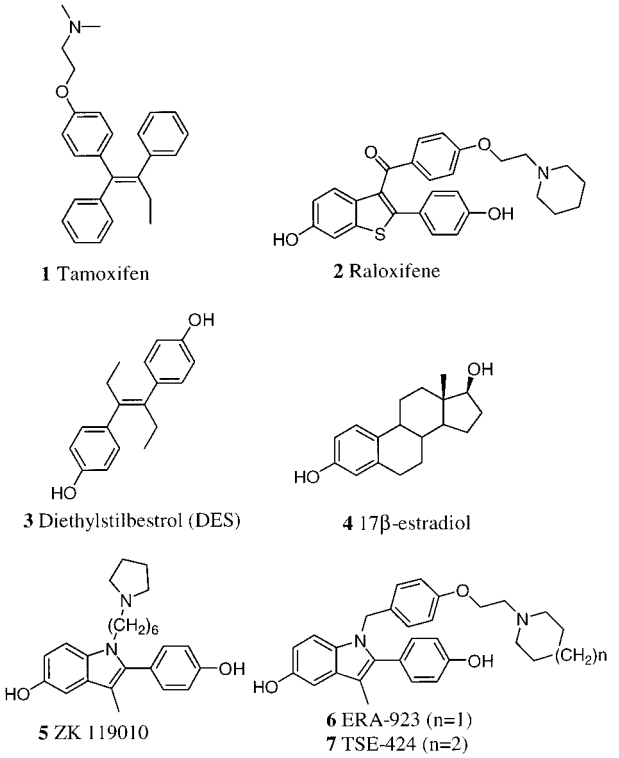

Figure 1. Structures of various estrogens.

This discovery has prompted a vigorous search for compounds with increasingly selective profiles.

Most of the selective estrogens reported to date share the common structural motif of a core recognition element consisting of two aryl groups separated by two atoms (often a stilbene type arrangement). Additionally, these compounds typically bear a third phenyl group containing a 4-aminoethoxy substituted phenyl group. It is believed that selective estrogens function by binding the ligand binding domain of the estrogen receptor through their stilbene-like cores and projecting the third phenyl group that contains the 4-aminoethoxy group into a region of space which corresponds to the 11 position of an estratriene nucleus (i.e., estrone or estradiol).[7] Indeed, X-ray cocrystallographic studies of 4-OH-tamoxifen (a high-affinity, active metabolite of tamoxifen) or raloxifene with the ligand binding domain of the estrogen receptor α (ERα) reveal this to be the case.[8,9] Moreover, when 4-OH tamoxifen or raloxifene bind the ligand binding domain of ERα, an altered receptor conformation is adopted where helix 12 is placed in a hydrophobic groove formed by residues from helices 3, 4, 5, and the turn connecting helices 3 and 4. This is in contrast to the situation with DES **3** (diethylstilbesterol, a nonsteroidal estrogen agonist) or 17β-estradiol **4** which both lack the amine-bearing side chain. When these estrogen agonists bind ER, helix 12 folds over, revealing a groove formed by residues from helices 3, 4, 5, and 12. This groove provides a binding

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10.1021/jm010086m CCC: $20.00
Published on Web 04/25/2001

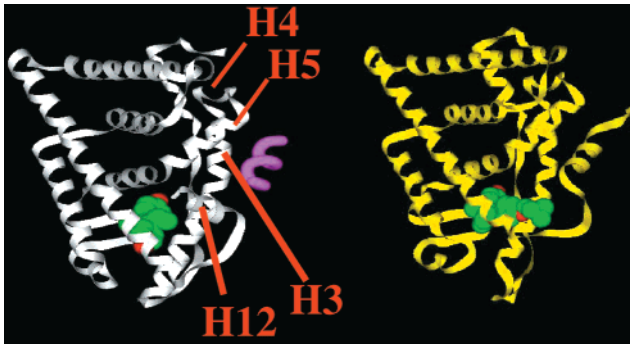


Figure 2. X-ray structures of the ligand binding domain of Era/DES (left) with a coactivator fragment (in purple), and the ligand binding domain of Era/4-OH tamoxifen (right).

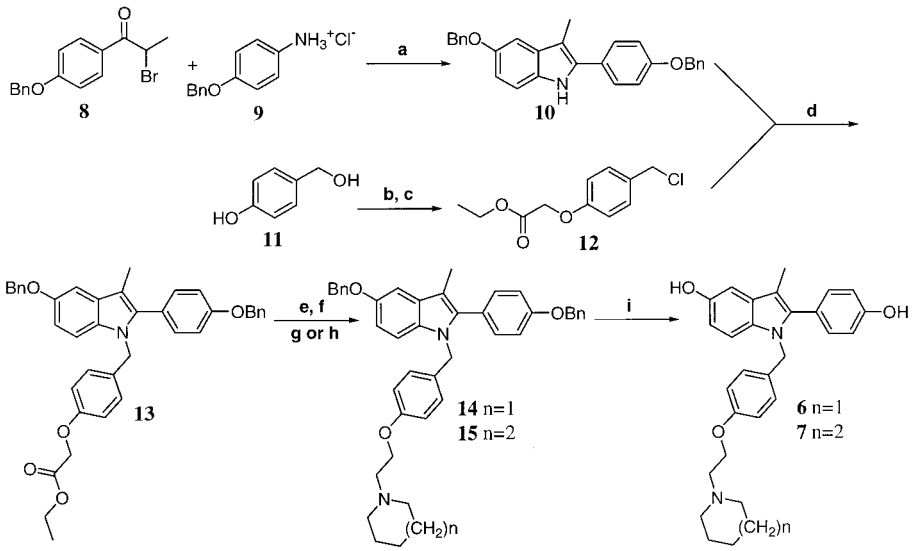
site for the LXXLL motif of various nuclear receptor coactivators. The difference in conformation of helix 12 results from the projection of the third phenyl group (in the case of a selective estrogen) containing the basic amine into the region of space which is normally occupied by helix 12 when in an agonist bound conformation (Figure 2).[9] The net result of these differences is that the altered conformation leads to a differential recognition between the receptor and several coregulatory proteins. It is believed, inter alia, that cell-specific regulation of target gene expression relies on differential expression or regulation of these various coregulatory proteins.

Since the stilbene-like cores of raloxifene (X-ray structure not shown), 4-OH-tamoxifen, and DES all locate in the ERα ligand binding domain in a similar fashion, it is reasonable to focus on the location and structure of the side chain as a fundamental determinant of selective estrogen action. In fact, it has been demonstrated that even very small changes in the side chain of raloxifene can result in large changes in its tissue selectivity profile.[10] On the basis of this premise, we were interested in examining the side chain SAR of certain estrogenic templates reported in the literature. Of particular interest was the 2-phenylindole compound, **5**.[11] Whereas it appears from inspection that the core template of the indole can function in a similar fashion as other selective estrogen stilbene-type cores, we postulated that the aliphatic, amine-containing side chain is too flexible to provide for optimal displacement of helix 12 upon binding. To that end, we decided to examine the effect of placing a rigidifying linker between the amine terminus and the indole nitrogen. To do this, we constructed a phenoxyethyl linker between the amine of the side chain and the N-1 position of the indole. In this way, the modified indoles have side chains that more closely resemble other selective estrogens already described. After various explorations around the core and side chain, we discovered ERA-923 **6** and TSE-424 **7**, both novel, highly selective estrogens with particularly non-estrogenic profiles on rat uterine tissue.

Chemistry. As shown in Scheme 1, the 3-methyl indole core **10** was synthesized in a relatively straightforward fashion from α-bromopropiophenone **8** and the aniline hydrochloride **9** via a Bischler-type indole synthesis.[12] We found the yields and reproducibility of this reaction could be increased by conducting the reaction in two steps but one pot. The side chain **12** was prepared by alkylation of commercially available 4-OH benzyl alcohol **11** with ethyl bromoacetate followed by conversion of the alcohol to the benzyl chloride **12** with $SOCl_2$ in THF. Alkylation of the indole **10** with the side chain **12** was accomplished with NaH in DMF. The ester **13** was subsequently reduced with LAH, and the primary alcohol thus produced was converted to the corresponding bromide by treatment with CBr_4 and triphenylphosphine. Substitution of this bromide with piperidine yielded **14**, and substitution with hexamethylenimine furnished **15**. Hydrogenation of **14** and **15** and conversion to their respective salts yielded **6** (HCl salt) and **7** (acetate salt).

Results and Discussion. Compounds **6** and **7** were evaluated for their ability to displace [^{3}H]17β-estradiol from estrogen receptor ligand binding domain constructs (human) of both the α- and β-receptors (ERα and ERβ).[13] The binding results are listed in Table 1 as IC_{50}s; values for unlabeled **5**, 17β-estradiol **4**, and **2** are included for

Scheme 1. Synthesis of Indoles **6** and **7**[a]



[a] Reagents and conditions: (a) Et_3N, DMF; (b) K_2CO_3, α-bromoethyl acetate; (c) $SOCl_2$, THF; (d) NaH, DMF; (e) $LiAlH_4$, THF; (f) TPP, CBr_4, THF; (g) piperidine, THF; (h) hexamethylenimine, THF; (i) H_2, Pd/C, EtOH/THF.

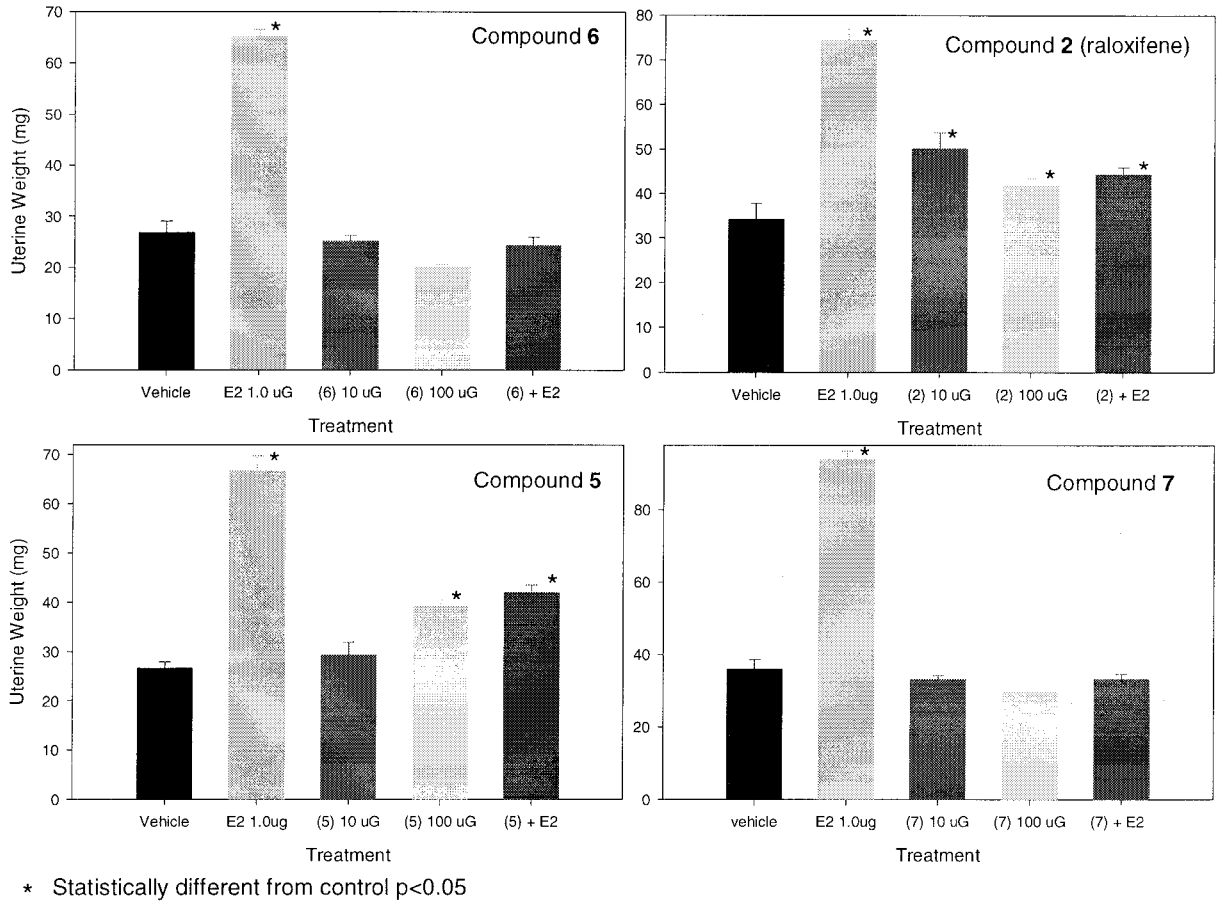


Figure 3. Uterine wet weight data from immature rats treated for 3 days subcutaneously. Neither **6** nor **7** demonstrate any increase in uterine weight when administered alone, and both completely antagonize 17β-estradiol stimulated wet weight gains. Conversely, raloxifene **2** statistically increased uterine wet weight at both 10 and 100 μg and did demonstrate antagonism of 17β-estradiol stimulated weight increase, but it converged with its 100 μg agonist activity. Compound **5** did exhibit agonist activity at 100 μg, with similar antagonist activity seen with raloxifene converging with its 100 μg effect. Doses administered are per animal.

Table 1. In Vitro Binding and MCF-7 Transfection Assay

	radioligand binding assay IC_{50}(nM)[a]		transient transfection assay in MCF-7 cells[a]	
	ERα	ERβ	tested as[b]	EC_{50} or IC_{50} (nM)[c]
3	3 ± 1	4 ± 2	agonist	0.007 ± 0.007
2	4 ± 3	43 ± 13	antagonist	0.72 ± 0.2
5	25 ± 13	242 ± 187	antagonist	69 ± 23
6	14 ± 10	40 ± 24	antagonist	1.5 ± 0.4
7	23 ± 15	85 ± 59	antagonist	3.7 ± 1.6

[a] n = average of at least two determinations (±SD). [b] Agonist mode run at 10 pM; antagonist mode run with compound against 10 pM 17β-estradiol. [c] EC_{50} refers to values in agonist mode; IC_{50} refers to antagonist mode.

comparison. As evidenced from the data in Table 1, both **6** and **7** show respectable affinity for both forms of the receptor, albeit with a slight preference for ERα. Consistent with our supposition that the core binding element generally drives basic binding affinity, compound **5** displays an affinity similar to that of **6** and **7** for the two receptors, while the benzothiophene **2** shows a high affinity for ERα but a somewhat weaker affinity for ERβ. Although understanding the distinct physiological role of ERα and ERβ is of considerable interest to the scientific community, at this time the consequence of a molecule's preferential binding to either receptor is not clear.

Compounds **6** and **7** were also evaluated for their functional estrogenic/antiestrogenic activity in an MCF-7 cell ERE-tk-luciferase (ERα mediated) assay (Table 1).[14] Since neither **6** nor **7** stimulate transcriptional activity in these cells, they were evaluated in the antagonist mode by competition with 10 pM 17β-estradiol. Only **6**, **7**, and **3** were potent antagonists in this assay; all having IC_{50}s less than 5 nM. It is interesting to note that while **5** displays a binding affinity for ERα similar to that of either **6** or **7**, it is not very potent in this MCF-7 ERE-tk luciferase assay that expresses only ERα. Perhaps, this is because the binding assay only utilizes the ligand binding domain of ERα while the transcriptional assay employs full length receptors with all domains intact. Alternatively, it may be due to other factors related to differential cellular uptake, metabolism, cellular transcription, or transactivation.[15] The generally poor correlation between ERα binding affinities and MCF-7 cell proliferation inhibition has been noted previously for a series of raloxifene analogues.[16]

Whether being used as a selective estrogen for prevention of osteoporosis or as an antiestrogen for breast cancer, it is critical that the candidate compound does not cause stimulation of the uterine endometrium in the target population, since estrogenic stimulation of the uterus leads to both an increase in uterine bleeding as well as an increase in the risk of uterine cancer. Thus, all compounds were tested in a 3-day immature rat

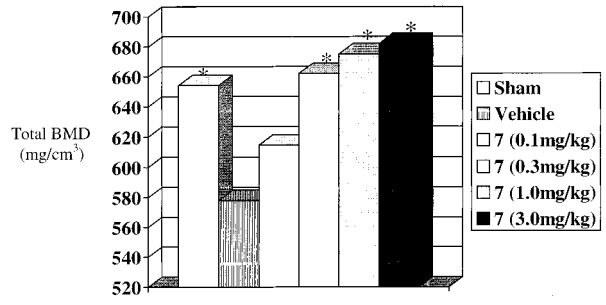


Figure 4. Total bone mineral density utilizing peripheral quantitative computerized tomography (pQCT) of the proximal tibia from ovariectomized rats treated with **7** orally for 6 weeks. TSE-424 demonstrates a significant effect from vehicle starting at 0.3 mg/kg.

uterine model.[13] Figure 3 demonstrates the comparative uterine stimulation of a subcutaneous administration of **2**, **5**, **6**, **7**, and **4**. Both **6** and **7** showed no significant uterine stimulation when dosed alone. In contrast, **2**, **5**, and **4** (E2 in Figure 3) all significantly increased the uterine weights of the treated animals, albeit it to different degrees. In the antagonist mode, **6** and **7** were able to completely inhibit the uterine wet weight increase effected by **4**. On the basis of this preclinical uterine data, we believe that **6** and **7** are among the most selective of estrogens reported to date.

In a 6-week ovariectomized rat study,[17] **7** was examined for its ability to prevent bone loss. Figure 4 demonstrates that **7** protected against bone loss in the proximal tibia and reached significant efficacy at a dose of 0.3 mg/kg per day. Additionally, **7** reduced total cholesterol with maximal efficacy (approximately 50%) at just 0.1 mg/kg per day while demonstrating no statistically significant effect on uterine wet weight in any of the treated animal groups.

From the data presented, side chain structure is an important determinant of selective estrogen action. Whereas the indole **5** displayed considerably reduced antiestrogen efficacy in an MCF-7 ERE-tk luciferase assay as well as some estrogen agonist activity in a 3-day immature rat uterine assay, compounds **6** and **7** behaved as potent antagonists in these assays. While it has been reported that varying the length of the aliphatic side chain can also affect the relative degree of estrogenicity/antiestrogenicity for a given compound in various cell-based systems, we believe that for the amine-containing side chains it is optimal to have the phenoxy group present to rigidify this portion of the molecule.[18]

Both **6** and **7** display very attractive selective estrogen profiles. Due to functional differences between the two compounds it was decided that **7** should be advanced for treatment of postmenopausal osteoporosis. Whereas **7** was very efficacious at protecting against bone loss and reducing total cholesterol in the ovariectomized rat model of osteopenia, **6** was a very potent and efficacious antiestrogen in various in vivo MCF-7 xenograft models.[19] Currently, **6** is in phase II clinical trials for the treatment of hormone-dependent metastatic breast cancer, and **7** has completed phase II clinical trials for the prevention and treatment of postmenopausal osteoporosis.

Supporting Information Available: Spectral and analytical data for all new compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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JM010086M