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Synthesis and Estrogenic Activities of Novel 7-Thiosubstituted Estratriene Derivatives

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Abstract—A diastereomerically pure series of 7α-thioestratrienes was prepared and evaluated for its affinity for both the human estrogen receptor α and the more recently discovered estrogen receptor β. The functional estrogenic activities of the compounds were measured in a MCF-7 ERE-tk-luciferase assay. The activities and selectivities of the compounds were sensitive to the nature of the thioether side chain.

Introduction

Recent years have witnessed the introduction of antiestrogenic compounds that are significantly devoid of any estrogenic activity.[1] It has been speculated that these compounds may offer an advantage over mixed estrogen agonists, such as tamoxifen, for the treatment of breast cancer. The partial agonism of tamoxifen has been implicated in the development of tamoxifen-resistant breast cancer as well as in the development of uterine cancer.[2,3] Compounds that have reduced estrogenic agonist profiles could provide a more complete estrogen withdrawal and thus might avoid the putative pitfalls of tamoxifen therapy. The prototypical, 'purely antiestrogenic' compounds were introduced by ICI and consist of estratrienes with long carbon chains connected to the steroid backbone at the 7α-position and possessing either amide (typified by ICI 164384 (**1** in Fig. 1)) or sulfoxide groups (typified by ICI 182780 (**2** in Fig. 1)).[4,5] Since then a number of compounds have been reported where similar functionality was attached to various positions of the estratrienes, including the 11β-position (**4** and **5** in Fig. 1)[6,7] as well as the 15 and 17 positions (not shown).[8] More recently, the connection of various alkanamide chains to the estratriene backbone at the 6 position through a sulfur atom has been reported (**6** in Fig. 1). Interestingly, the 6-thiosubstituted estratriene compounds were reported to show no antiestrogenic activity in an estrogen receptor positive ZR-75-1 cell line.[9] So far, with respect to antiestrogens having the steroidal estratriene core, only the attachment of groups at the 7α-position and the 11β-position have been conducive to the generation of compounds with 'pure antiestrogen' characteristics.

We were interested in investigating the effect of connecting various antiestrogenic functionalities by a sulfur atom at the 7-position of the estratriene backbone. We envisioned the synthesis of compounds that contained various moieties connected to the sulfur atom that would resemble the functionality of other known steroidal and antisteroidal antiestrogens (Fig. 2). It has been suggested that nonsteroidal antiestrogens bind to the estrogen receptor through their stilbene like cores, and project a functionality (usually a 4-aminoethoxy phenyl group (i.e. tamoxifen, raloxifene, centchroman, etc.)) into a region of space that corresponds to the 7α-position or 11β-position of an estratriene nucleus.[10] Indeed, X-ray crystal structures of ERα with 17β-estradiol and raloxifene or tamoxifen show that the aminoethoxy side chains of the nonsteroidal compounds project from the region of space which corresponds to the 11β-position of 17β-estradiol.[11,12]

Estratrienes with a phenylaminoethoxy group placed in the 7α-position or the 11β-position have been synthesized and reported (compounds **3** and **4** in Fig. 1).[13] We

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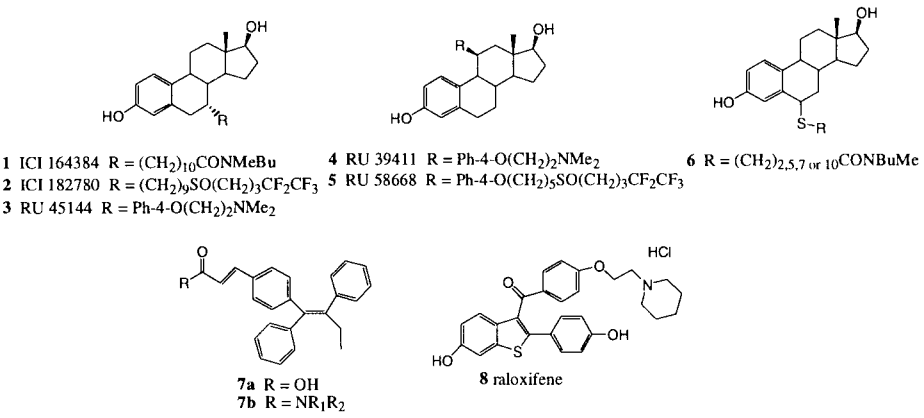


Figure 1. Structures of some steroidal and nonsteroidal antiestrogens.

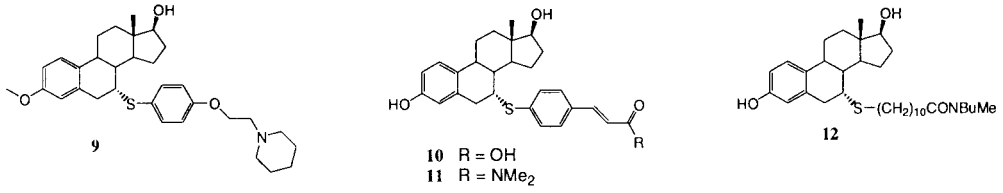


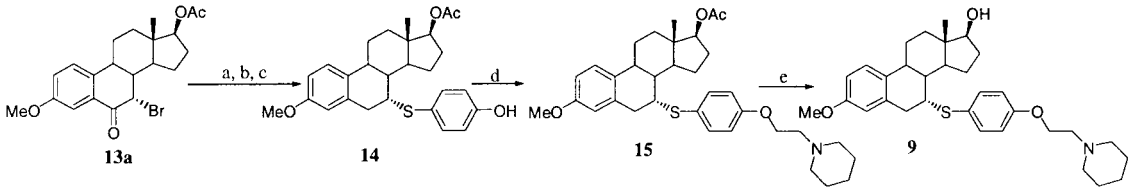
Figure 2. 7α-Thiosteroids with various antiestrogenic functionalities attached.

thought it would be informative to probe the 7α-region of the estratriene nucleus by connecting the phenyl group to the steroid backbone by a sulfur atom and thus provide a 'hinging' atom to change the orientation of the phenyl group in the antiestrogenic effector space of the estrogen receptor. Compound **9** shown in Figure 2 contains the 4-(piperidinoethoxy)phenyl group present in the tissue selective estrogen, raloxifene (compound **8** in Fig. 1). In the ovariectomized rat model, raloxifene (Evista® for osteoporosis in humans) is antiestrogenic on uterine and breast tissue while showing estrogen agonist-like effects in bone and the cardiovascular system.[14] The tissue selective estrogenicity/antiestrogenicity of this benzothiophene template is very sensitive to the nature of the side chain appended to the 3-position. It appears that the 4-piperidinoethoxy side chain is optimal for reducing estrogen agonism on the uterus.[15]

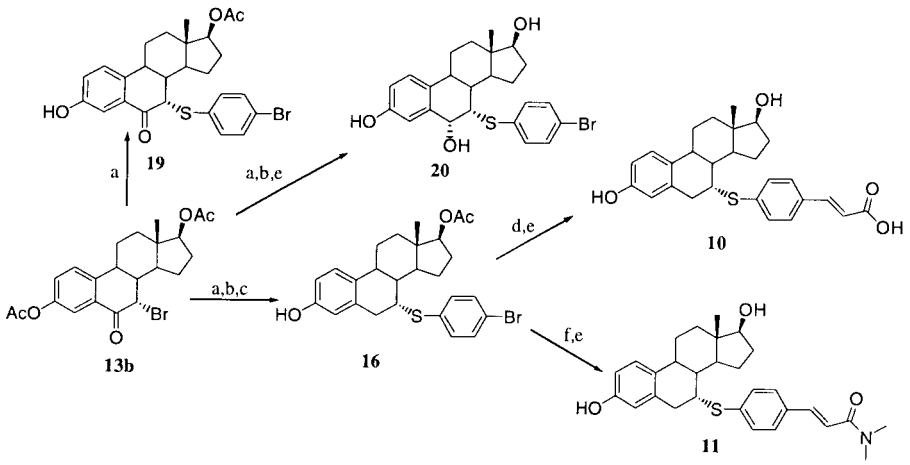
Analogues of tamoxifen have been reported where the aminoethoxy group is replaced by an acrylamide or acrylic acid moiety (compounds **7a** and **7b** in Fig. 1).[16] These compounds were reported to show reduced uterine activity relative to tamoxifen while still displaying the tissue selective, bone sparing activity of tamoxifen in an ovariectomized rat assay. Compounds **10** and **11** (Fig. 2) were synthesized in order to explore the effects of the acrylamide or acrylic acid substituents when placed at the 4-position of the thiophenol substituted at the 7-position of the estratriene. Finally, we investigated the effect of an ICI 164384-like undecamide side chain attached to the estratriene nucleus through the tethering sulfur atom (compound **12** in Fig. 2).

Chemistry

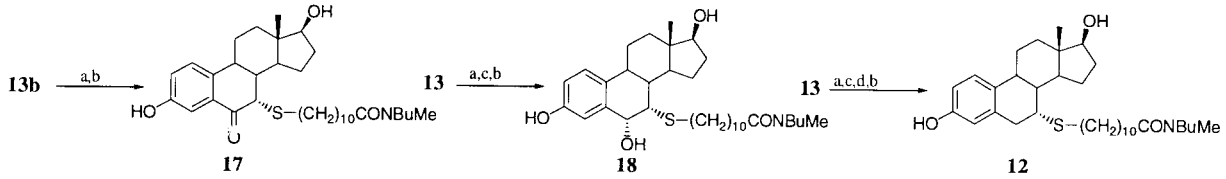
The synthesis of the various analogues shown in Figure 2 is demonstrated in Schemes 1–3. Analogue **9** shown in Scheme 1 was prepared by substituting the bromoketone **13a**[17] with 4-OH-thiophenol (only alpha isomer detected) followed by reduction of the ketone with $NaBH_4$ and subsequent deoxygenation with TFA/Et_3SiH. The observation of a single diastereomer can probably best be explained by substitution followed by full epimerization under the reaction conditions.[18] The basic side chain was attached by reaction of **14** with β-chloroethylpiperidine and **9** was obtained after base hydrolysis of the 17-acetyl group.



Scheme 1. Synthesis of 7α-thiophenol-4′-piperidinoethoxy estratriene. (a) 4-OH thiophenol, NaH, DMF; (b) $NaBH_4$, EtOH; (c) TFA, Et_3SiH; (d) β-chloroethylpiperidine, K_2CO_3, DMF; (e) NaOH, MeOH/H_2O.



Scheme 2. Synthesis of 7α-thiophenyl-4′-acrylamide and 7α-thiophenyl-4′-acrylic acid estratrienes. (a) 4-Bromothiophenol, NaH, DMF; (b) $NaBH_4$, EtOH; (c) TFA, Et_3SiH; (d) ethyl acrylate, $Pd(OAc)_2$, P(o-tol)$_3$, Et_3N; (e) NaOH, MeOH/H_2O; (f) *N*,*N*′ dimethyl acrylamide, $Pd(OAc)_2$, P(o-tol)$_3$, Et_3N.



Scheme 3. Synthesis of 7α-thioundecamide estratrienes. (a) HS-$(CH_2)_{10}$CONBuMe, NaH, DMF; (b) NaOH, MeOH/H_2O; (c) $NaBH_4$, EtOH; (d) TFA, Et_3SiH.

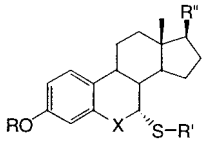
The analogues containing the acrylic acid and dimethyl acrylamide moiety (compounds **10** and **11** in Scheme 2) were prepared by substitution of the bromoketone **13b**[17] with 4-bromothiophenol and deoxygenation at the 6-position as previously described. The acrylic acid **10** was prepared by Heck coupling of the substrate **16** with methyl acrylate followed by saponification. The dimethyl acrylamide (**11**) was prepared by an analogous reaction of **16** with *N*,*N*′-dimethylacrylamide followed by saponification. The bromo compounds **19** and **20** were prepared as shown and tested as well. Finally, the thioundecamide analogues **12**, **17** and **18** were prepared as shown in Scheme 3. The thioundecamide side chain was prepared as previously described by Labrie.[19]

Discussion of Biological Evaluation of Estrogenic Activity

The structures of the compounds synthesized and tested are shown in Table 1. The compounds were first tested for their ability to displace [^{3}H]17β-estradiol from estrogen receptor ligand binding domain constructs of both the alpha and beta receptors.[20] The binding results are listed in Table 2 as IC_{50}s and a value for unlabelled 17β-estradiol is included for comparison. None of the steroids tested were particularly selective for one receptor over the other although compound **20** showed an approximately 6-fold preference for the beta receptor. Some of the compounds displayed reasonably high affinities for both receptors. Optimal binding in the series was achieved when the 6-position was unsubstituted (compare **12** with **17** and **18**). This is consistent with the reported data for 6-oxo-estradiol when compared to 17β-estradiol.[21] The lack of a free phenol in compound **9** is a likely explanation for its poor receptor affinity. The functional estrogen receptor α activity of these compounds was determined using the MCF-7 ERE-tk-luciferase assay and was first tested in an antagonist mode (by cotreating with 0.1 nM 17β-estradiol) to generate an IC_{50}.[22]

Compounds that did not block the effect of 17β-estradiol were tested alone to generate an EC_{50}. Interestingly, the compounds that contained a phenyl linker failed to function as antagonists and demonstrated only estrogen agonist-like activity in the MCF-7 cells. This is despite the fact that they contain similar functionality to known nonsteroidal antiestrogens shown in Figure 1 (compare steroid analogue **10** to tamoxifen analogue **7a** and steroid analogue **9** to raloxifene **8**). Despite showing no activity in the binding assay, compounds **9** and **19** showed agonist activity in the MCF-7 cells. Presumably this is due to metabolic activation at the 3 or 17 position. Compounds that contain the thioundecamide side chain behaved as estrogen antagonists (compounds **12**, **17** and **18**). This demonstrates that the sulfur atom located at the 7α-position is not per se a barrier to achieving high receptor affinity and potent estrogen antagonism. This is in contrast to the report that no significant estrogen antagonism was displayed when similar thio-alkanamide side chains were placed at the

Table 1. Compound numbers and structures



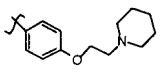
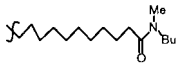
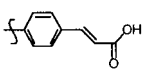
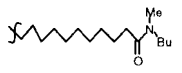
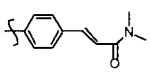
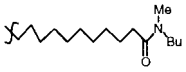
Compound	R	X	R′	R″	Compound	R	X	R′	R″
9	CH_3	CH_2		OH	**17**	H	C=O		OH
10	H	CH_2		OH	**18**	H	CH–OH		OH
11	H	CH_2		OH	**19**	H	C=O		OAc
12	H	CH_2		OH	**20**	H	CH–OH		OH

Table 2. Activity of compounds in binding and reporter gene assays

	Radioligand binding assay[a]		ERE/MCF-7 Transient transfection assay (ER-α)[b]	
Compound	ER-α IC_{50} (nM)	ER-β IC_{50} (nM)	Antagonist IC_{50} (nM)	Agonist EC_{50} (nM)
17β-estradiol	3 ± 1	4 ± 2	Not active	0.007 ± 0.007 (100)
ICI-182780	6 ± 1	7 ± 3	0.47 ± 0.2 (100)	Not active
Raloxifene	2 ± 1	43 ± 13	0.72 ± 0.2 (100)	Not active
7a[c]	39	56	654	Not active
9	Not active	Not active	Not active	336 ± 20 (116)
10	46 ± 23	21 ± 7	Not active	13 ± 1 (104)
11	94 ± 55	59 ± 40	Not active	173 ± 6 (104)
12	9 ± 4	9 ± 4	3.5 ± 2 (99)	Not active
17	33 ± 18	37 ± 8	519 ± 9 (97)	28 ± 8 (51)
18	32 ± 21	32 ± 21	316 ± 52 (98)	~40 (23)
19	Not active	Not active	Not active	5 ± 0 (105)
20	397 ± 143	63 ± 17	Not active	7 ± 1 (78)

[a]Mean ± S.D.; $n \geq 2$ unless noted.
[b]Mean ± S.D.; $n \geq 2$ unless noted; efficacy in parentheses.
[c]$n = 1$; Not active means no effect or competition at < 1000 nM.

6-position of the estratriene nucleus.[9] When compound **12** was dosed alone in a 3-day immature rat uterine assay, it did not stimulate uterine wet weight gain. When dosed with 17β-estradiol, compound completely and potently blocked the uterine weight gain normally seen when dosing 17β-estradiol alone.[23] This is consistent with the supposition that compound **12** behaves as a pure antiestrogen in the same way as its progenitor, ICI 182780 (compound **1** in Fig. 1), does in these assays.

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20. Binding material and methods: A crude lysate of *E. coli* expressing the ligand binding domain (D, E, & F) of human ERα or ERβ was prepared. Both receptors and compounds were diluted in 1X Dulbecco's PBS (DPBS) supplemented with 1 mM EDTA. Using a high binding masked microtiter plate, 100 μL of receptor (1 μg/well) was combined with 2 nM [^{3}H]-17β-estradiol and various concentrations of compound. After between 5 and 15 hours at room temperature, the plates were washed with DPBS/1mM EDTA and bound radioactivity determined by liquid scintillation counting. The IC_{50} is defined as the concentration of compound which decreases total [^{3}H]-17β-estradiol by 50%.
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23. Procedure: Sprague–Dawley rats ($n = 6$, 18 day old) were treated by daily subcutaneous injection with compounds or vehicle (50% DMSO/50% DPBS) for three days. On day 4 the animals were euthanized by CO_2 asphyxiation. Uteri were weighed after removing external fat and expressing any luminal fluid.

Compound dose (μg/rat/day)	Compound **12** Mean uterine weight (mg) ± stdev	Tamoxifen Mean uterine weight (mg) ± stdev
1	31.8 ± 3.4	53.3 ± 1.5
10	33.6 ± 3.5	69.6 ± 1.9
100	41.6 ± 2.1	71.4 ± 1.6
100 + estradiol (1μg)	46.0 ± 4.1	Not tested
	veh = 43.1 ± 4.7; estradiol (1μg) = 82.1 ± 4.1	veh = 42.7 ± 4.4; estradiol (1μg) = 98.2 ± 10.4