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Estradiol derivatives bearing sulfur-containing substituents at the 11β or 7α positions: versatile reagents for the preparation of estrogen conjugates

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Abstract—Estradiol derivatives bearing HS-, $HSCH_2$-, $HSCH_2CH_2$-, MeS-, $MeSCH_2$-, $MeSCH_2CH_2$-, or $PhCH_2SCH_2CH_2$-groups at the 11β position or an HS-group at the 7α position have been synthesized, and their binding affinity to the estrogen receptor (ER) determined. Nearly all of these substituted estrogens retain high binding affinity, and at the 11β position, the sulfur atom has an effect on ER binding that is similar to that of a carbon atom. These thiol derivatives are promising intermediates for the preparation of a variety of estradiol conjugates. The methyl sulfides, in particular, might potentially be developed as ^{11}C-labeled agents for imaging ER-positive tumors by positron emission tomography.

1. Introduction

There is great interest in the development of estrogens having tissue-selective pharmacological profiles that are optimal for hormone replacement therapy and breast cancer prevention and treatment, in particular, ones that would block the proliferative actions of estrogens in the uterus and breast, but maintain beneficial estrogen function in the bone, brain, and the cardiovascular system.[1–3] There is also interest in the development of radioactive estradiol derivatives that can be used as receptor-based agents for imaging or radiotherapy of estrogen-responsive breast cancer.[4] Both of these goals have stimulated continued interest in the study of the relationship between the structure of ligands for the estrogen receptor (ER) and their estrogenic activity and ER binding affinity.

Nonsteroidal estrogens have been actively investigated, particularly in the search for selective modulators of estradiol receptors, because of the ease with which nonsteroidal structures can be chemically manipulated and altered to optimize their binding and activity properties.[5] In fact, several nonsteroidal estrogens, including tamoxifen and raloxifene, are termed selective estrogen receptor modulators or SERMs. Continued investigation of nonsteroidal compounds is encouraged by the estrogenic activity of phytoestrogens and other nonsteroidal natural products, such as coumestrol, genistein, and β-zearalenone, compounds of polyketide or mixed biosynthetic origin. Despite the popularity of nonsteroidal ER ligands, we thought that the search for new SERMs and radiotracers or radiotherapeutic agents might greatly benefit from the possibility of generating easily a wide variety of steroidal derivatives, namely, estrogens substituted at the 11β or 7α positions. These positions, although more difficult to functionalize than the 17α position—which has been the most extensively adopted for belling the estradiol molecule—are more tolerant of the presence of substituents, which can frequently give rise to derivatives having high binding affinity, as well as potent estrogenic or antiestrogenic activity.[6]

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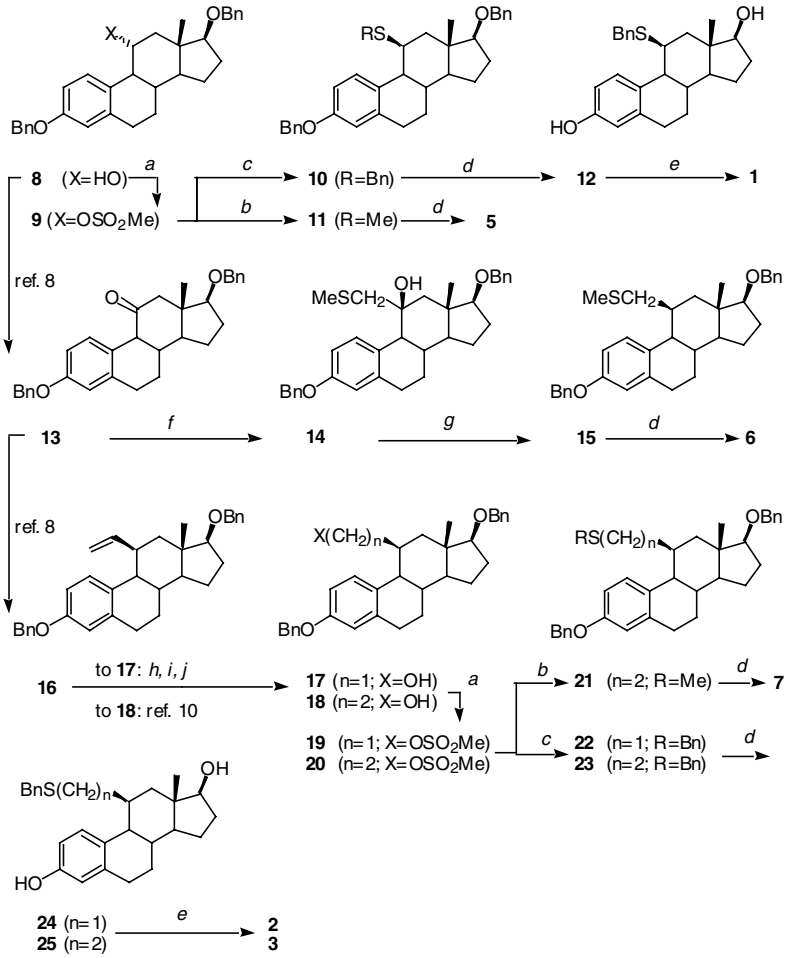
In this report, we describe the synthesis of estradiol compounds **1**–**4**, steroids that are mercapto substituted at the 11β or 7α positions. Because of the ease with which carbon–sulfur bonds can be formed and the stability of this bond, these compounds are promising intermediates for generating estradiol conjugates of different types. For instance, alkylation of SH with long chain substituents could lead to sulfur analogs of important antiestrogens; alkylation with groups containing a triple bond, a cyclopentadiene, or a macrocyclic ligand could give rise to compounds amenable for conjugation with transition metals.[7] Besides 11-substituted mercapto derivatives **1**–**3**, we also report the synthesis and ER binding properties of their respective methyl sulfides **5**–**7**. The ER binding data on these compounds reveals the influence of the sulfur atom on the binding properties of estradiol derivatives, and they show that compounds **5**–**7**, which could be readily labeled with the positron-emitting radionuclide carbon-11 by the reaction of suitably protected versions of the corresponding thiol compounds **1**–**3** with C-11 methyl iodide, are promising new candidates as receptor-based agents for imaging ER-positive breast tumors using positron emission tomography (PET). It is of note that these compounds would have the radiolabel at a site where it is easily introduced and well tolerated (if not binding enhancing), and it would leave positions 15, 16, and 17 free to allow for substitution to control metabolism and clearance as is needed to achieve effective target to background contrast with an imaging radiopharmaceutical labeled with an isotope having a half life of 20.3 min.

2. Results and discussion

2.1. Chemical synthesis

2.1.1. Synthesis of 11β-substituted estrogens. The estradiol derivatives with sulfur-containing substituents at 11β (**1**–**3** and **5**–**7**) were prepared as outlined in Scheme 1. The hydroxy derivative **8**, a common reaction intermediate, was obtained from commercially available estrone through the following steps, as detailed elsewhere:[8] (a) dehydrogenation with dichlorodicyanoquinone; (b) reduction with sodium borohydride; (c) di-benzylation with benzylbromide and sodium hydride, and (d) hydroboration followed by oxidation with alkaline peroxide. To obtain compounds **1** and **5**, in



Scheme 1. Conditions: (a) $MeSO_3Cl$, Py. (b) CH_3SNa, DMF, 60 °C. (c) $PhCH_2SNa$, DMF, 60 °C. (d) BBr_3, CH_2Cl_2, −5 °C. (e) Na/EtOH, Δ. (f) CH_3SCH_2Li/TMEDA. (g) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2. (h) OsO_4, 4-methylmorpholine *N*-oxide, CH_3COCH_3. (i) $NaIO_4$, SiO_2, H_2O/CH_2Cl_2. (j) $NaBH_4$/MeOH.

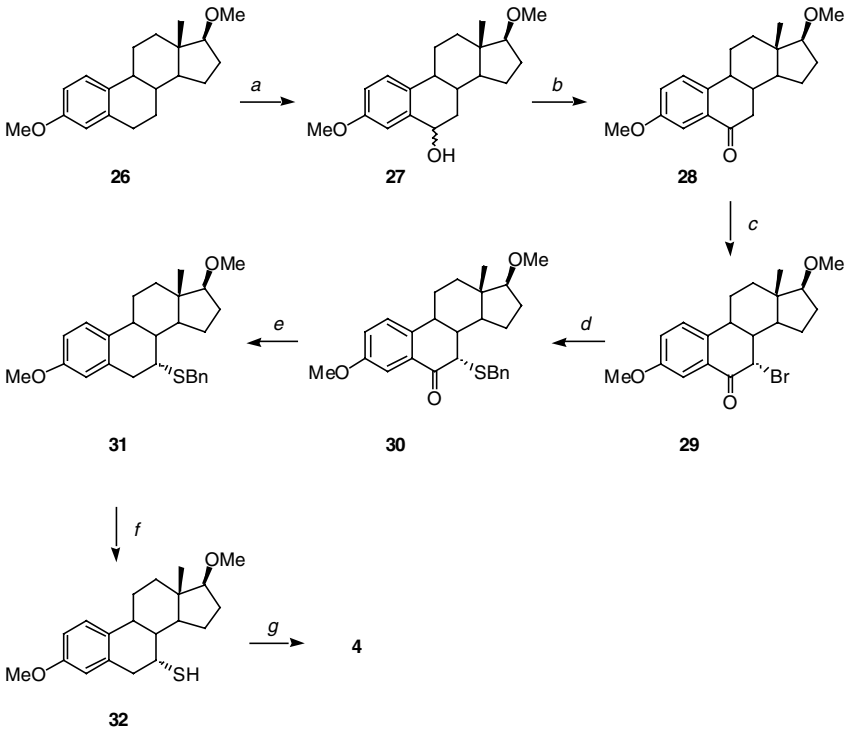
which the sulfur atom is directly bound to the 11β-position, alcohol **8** was converted to the corresponding methanesulfonate **9**, which underwent clean displacement by sodium benzylmercaptide to give the tribenzylated derivative **10**. The *O*-benzyl groups were removed with boron tribromide, and the resulting S-benzylated derivative **12** was further deprotected by reaction with excess of sodium in ethanol, thus yielding the parent mercaptan **1**. Methyl sulfide **5** was obtained from mesylate **9** by reaction with sodium methylmercaptide (prepared by reduction of dimethyl disulfide with sodium powder in tetrahydrofuran), followed by debenzylation with boron tribromide of intermediate dibenzylated derivative **11**.

The higher methyl sulfide **6** was obtained from ketone **13** by an extension of our recent approach to 11β carbon-substituted estradiol derivatives.[8] Thus, the lithium derivative of dimethyl sulfide (obtained by hydrogen metal exchange with butyllithium in the presence of tetramethylethylenediamine)[9] added to the carbonyl of **13** the αface, as is generally observed with other nucleophiles, to give the tertiary alcohol **14**. This material underwent ionic reduction with triethylsilane and boron trifluoride etherate, leading, after O-debenzylation of the intermediate ether **15**, to the expected sulfide **6**. The 11β stereochemistry of **6** was confirmed by an alternative synthesis from **16** in which the stereochemistry at 11 is known (vide infra).

Attempts to generate mercaptan **2** from sulfide **6** by a sequence involving oxidation to a sulfoxide and subsequent Pummerer rearrangement of the sulfoxide did not result in a clean transformation, and the following alternative route from the vinylestradiol **16** was preferred. Thus, the unsaturated derivative **16** was dihydroxylated with osmium tetroxide and morpholine *N*-oxide; the crude diol was cleaved with sodium periodate, and the intermediate aldehyde, without purification, was subjected to sodium borohydride reduction to afford the hydroxymethyl derivative **17**. This material was converted to mercaptan **2** by the sequence involving: (a) formation of the mesylate **19**; (b) mesylate displacement by sodium benzylmercaptide to give **22**; (c) O-debenzylation with boron tribromide to give diol **24**, and finally (d) S-debenzylation. Treatment of mesylate **19** with sodium methylmercaptide gave a sulfide identical to compound **15** obtained from precursor **14**, thus confirming the 11β stereochemistry of **15**.

For the synthesis of the highest mercaptan and methylsulfides of the series, olefin **16** was converted to mesylate **20** through alcohol **18**, as already reported.[10] The reaction of **20** with either sodium methylmercaptide or benzylmercaptide gave sulfides **21** or **23**, respectively. Debenzylation with boron tribromide converted the ethers **21** and **23** to the alcohols **7** and **25**, respectively. Further debenzylation with sodium in ethanol converted **25** to the mercapto diol **3**.

2.1.2. Synthesis of 7α-substituted estrogen. The synthesis of 7α-mercaptoestradiol **4** is outlined in Scheme 2. Estradiol was protected as the dimethylether **26** and hydroxylated at C-6 to give alcohol **27**, according to our previously reported procedure involving deprotonation



Scheme 2. (a) 1. LDA/*t*-BuOK; 2. $B(OCH_3)_3$; 3. H_2O_2. (b) PCC, CH_2Cl_2. (c) Br_2, CH_2Cl_2. (d) Conditions A: BnSH/*t*-BuOK, Δ. Conditions B: BnSH/NaOH. (e) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2. (f) Na/EtOH. (g) $AlBr_3$/EtSH.

of the 5-H, quenching of the anion with trimethyl borate, and oxidation of the intermediate boronoic acid with hydrogen peroxide.[11] An improved procedure for the preparation of **27** is here reported (involving the use of potassium *tert*-butoxide as the additive and butyllithium with a catalytic amount of diisopropylamine as the metalating agent), which leads to higher and more consistent yields of product while saving lithium reagents (see Experimental). The alcohol **27** was oxidized to the ketone **28**, which underwent clean bromination to give 7α-bromoketone **29** as the sole (probably kinetically and thermodynamically controlled) product.[12] Exposure of this bromoketone (**29**) to the potassium salt (or sodium salt) of benzylmercaptan gave the 7α sulfide **30** (retention of configuration) as the thermodynamically controlled product, the result of substitution and then epimerization, presumably via enol formation.

Ionic reduction of keto sulfide **30** gave the sulfide **31** stereospecifically and in high yield. Debenzylation of **31** (sodium in ethanol) followed by the demethylation ($AlBr_3$ in methylene chloride) of the intermediate thioether **32** gave the 7α-mercaptoestradiol **4**.

2.2. Binding affinity determination

The binding affinities of the sulfur-containing estrogen analogs for the estrogen receptor were determined by a competitive radiometric binding assay using lamb uterine cytosol as a source of receptor (see Experimental). Affinities are expressed relative to that of the tracer, [^{3}H]estradiol, as relative binding affinity (RBA) values, where the RBA of estradiol is 100. RBA values are measured at both 0 and 25 °C. Receptor binding assays are traditionally run at 0 °C, but some compounds show considerably higher binding affinities at 25 °C for reasons that are not well understood: this may have to do with achieving full equilibration in the competitive binding assay,[13] or accentuating of the hydrophobic component of the binding affinity.[14] The values at 25 °C may be more relevant to the behavior of these compounds as radiopharmaceuticals for in vivo imaging.[15]

The RBA values of the new sulfur-containing estradiol derivatives, described above, are shown in Table 1. Introduction of an SH at the position 11β of estradiol results in a derivative (**1**) that has comparable to improved binding properties compared to the parent ligand. Since the 11β-hydroxy analog of **1** has a lower RBA than estradiol,[6a] the closer resemblance of **1** to 11β-methylestradiol can be rationalized on the basis of the relatively nonhydrophilic nature of the mercapto group. A further improvement in binding affinity is observed going to the higher homologues, **2** and **5**, in which the 11β substituent consists of the mercaptomethyl or the methylmercapto group, respectively: the higher affinity of these two isomers is the thioether (**5**). It is worth noting that the affinities of analogs **2** and **5** are somewhat less than those observed for the isosteric estradiol derivatives bearing an 11β-chloromethyl (110/1780 at 0/25 °C), ethyl (133/1372 at 0/25 °C lamb), or vinyl group (117/1780 at 0/25 °C lamb), but superior to those of the oxygenated analog 11β methoxyestradiol (8.5/86 at 0/25 °C lamb).[6a] Higher homologues with a sulfur containing three-atom substituent at 11β (**3** and **6**) have significantly lower RBA than their parents: isomer **3**, having a terminal mercaptan, has an RBA value that is lower than that for estradiol, but the thioether isomer **6** has RBA values that are comparable to higher than that of estradiol. Methylation of the SH group of **3** produces the compound (**7**) that has the lowest affinity in the series. Curiously, however, benzylation produces a derivative (**25**) having better binding properties than the methyl analog **7**. It is not clear why some compounds (**1**, **2**, **5**, and **6**) show a pronounced temperature dependence in their ER binding affinity, whereas others (**3**, **7**, and **25**) do not, although such observations have been made by us and others previously.[13–15] The introduction of the mercapto group at the 7α position substantially depresses the binding affinity, as generally observed with other groups larger than a methyl, and the temperature dependence of the binding (lower at higher temperature) is also different for what observed for the introduction of the mercapto group at the 11β position.

Table 1. Relative binding affinity of sulfur-containing estradiol derivatives

Compd	Substituent	RBA (0 °C)	RBA (25 °C)
Estradiol	H-	100	100
1	HS-11β	49 ± 7	270 ± 1
5	MeS-11β	29 ± 4	290 ± 15
2	$HSCH_2$-11β	57 ± 22	550 ± 20
6	$MeSCH_2$-11β	65 ± 4	140 ± 30
3	$HSCH_2CH_2$-11β	40 ± 1	57 ± 20
7	$MeSCH_2CH_2$-11β	31 ± 7	19 ± 2
25	$PhCH_2SCH_2CH_2$-11β	110 ± 20	100 ± 20
4	HS-7α	43 ± 0	5 ± 1

3. Conclusion

In conclusion, sulfur-containing groups have been introduced into positions 11β and 7α of estradiol. The sulfur atom promotes high estrogen receptor binding affinity when it is part of a small group at 11β, but the mercapto group depresses the binding when present in the 7α position. The mercapto derivatives should be convenient, advanced intermediates for the preparation of a various estradiol conjugates that could be used for different purposes. The methylthio derivatives **5** and **6** are promising candidates as ^{11}C labeled receptor-based imaging agents for ER-positive breast cancer.

4. Experimental

Melting points were taken with a Kofler hot plate apparatus and are uncorrected. For analytical TLC Merck Silica Gel F-254 on aluminum plates were used; for the visualization of spots, the plates were soaked with an ethanol solution containing phosphomolybdic

acid (5%) and sulfuric acid (5%), and heated with a heat gun. For column chromatography, the technique described by Still and Kahn,[16] was adopted, using mixture of hexane and EtOAc or CH_2Cl_2 as eluants. ^{1}H NMR (200 MHz) spectra were obtained using a Bruker AC 200 spectrometer for samples dissolved, unless otherwise indicated, in $CDCl_3$; chemical shifts (δ) are in parts per million downfield from tetramethylsilane as internal standard. Elemental analyses were performed by microanalytical laboratory of the Faculty of Pharmacy of the University of Pisa. All the reactions involving organometallic reagents were performed under nitrogen in solvents distilled from sodium benzophenone ketyl. Unless otherwise stated, solutions were dried with magnesium sulfate and evaporated in a rotary evaporator under diminished pressure. Compounds **8**, **13**, **16**, **18**, and **20** were synthesized as previously reported.[8,10]

4.1. 3,17β-Bis(benzyloxy)-11α-methanesulfonylestra-1,3,5(10)-triene (9)

To a solution of **8** (0.1 g, 0.21 mmol) in methylene chloride (0.5 mL) were added pyridine (1 mL, 12 mmol) and methanesulfonyl chloride (0.5 mL, 6 mmol), and the mixture was stirred at room temperature for 18 h. Ice was added, and reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with 5% aq HCl, dried, and evaporated to give a residue from which **9** (0.1 g, 95% yield) was obtained by trituration with ether as a crystalline solid. Mp 145–147 °C. ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 3 H, 18-H), 3.0 (s, 3 H, CH_3–SO_3), 3.55 (t, $J = 8$ Hz, 1H, 17-H), 4.52, 5.01 (2s, 2×2H, PhCH_2), 5.38 (m, 1H, 11-H), 6.74 (d, $J = 2.6$ Hz, 1H, 4-H), 6.86 (dd, $J = 2.6$ and 8.5 Hz, 1H, 2-H). EIMS m/z 546.2442 (calcd for $C_{33}H_{38}O_5S$ 546.2440).

4.2. 3,17β-Bis(benzyloxy)-11β-benzylthioestra-1,3,5(10)-triene (10)

Benzyl mercaptan (1.4 mL, 12 mmol) was added dropwise to a suspension of NaH (50% dispersion in oil, 0.3 g, 6 mmol) in dry DMF (1 mL), followed by the addition of **9** (840 mg, 1.53 mmol). The mixture was stirred at 60 °C for 18 h. The excess of NaH was destroyed by addition of water, and then the mixture was partitioned between water and Et_2O. The organic layer was washed with water, dried, and evaporated to leave a residue from which **10** (640 mg, 73% yield) was obtained by chromatography (9.5:0.5, hexane–EtOAc) as a glass. ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3H, 18-H), 3.45 (m, 2H, 11-H, 17H), 3.67 (s, 2H, PhCH_2S), 4.52, 5.0 (2s, 2×2H, PhCH_2), 6.6 (m, 3H, 1-H, 2-H, 4-H), 7.3 (m, 15H, *Ph*CH_2). EIMS m/z 574.2906 (calcd for $C_{39}H_{42}O_2S$ 574.2905).

4.3. 11β-Benzylthioestra-1,3,5(10)-triene-3,17β-diol (12)

BBr_3 (2 mL, 1 M in CH_2Cl_2) was added dropwise to a cooled (−5 °C) solution of **10** (230 mg, 0.4 mmol) in methylene chloride. After 45 min stirring at 0 °C, the mixture was partitioned between methanol and EtOAc. The organic layer was washed with 10% aq sodium bicarbonate and then with brine, dried, and evaporated to leave a residue from which **12** (0.1 g, 63% yield) was obtained by chromatography (2:1, hexane–EtOAc) as a glass. ^{1}H NMR (CD_3OD) δ 1.03 (s, 3H, 18-H), 3.55 (s, 2H, PhCH_2S), 6.4 (m, 3H, 1-H, 2-H, 4-H), 7.15 (m, 5H, *Ph*CH_2S). EIMS m/z 394.1967 (calcd for $C_{25}H_{30}O_2S$ 394.1966).

4.4. 11β-Mercaptoestra-1,3,5(10)-triene-3,17β-diol (1)

Sodium (300 mg, 13 mmol) was added in small portions to a refluxing solution of **12** (80 mg, 0.2 mmol) in EtOH (50 mL); complete consumption of **12** was checked by TLC. Diluted HCl was added under nitrogen, and the excess of EtOH was evaporated. The residue was partitioned between water and EtOAc. The organic layer was dried and evaporated to give a residue from which **1** (40 mg, 66% yield) was obtained by trituration with ether as a crystalline solid. Mp 228–230 °C. ^{1}H NMR (CD_3OD) δ 1.04 (s, 3H, 18-H), 3.64 (m, 1H, 17-H), 4.01 (m, 1H, 11-H), 6.50 (d, $J = 2.6$ Hz, 1H, 4-H), 6.60 (dd, $J = 2.6$, 8.4 Hz, 1H, 2-H), 6.92 (d, $J = 8.4$ Hz, 1H, 1-H). EIMS m/z 304.1501 (calcd for $C_{18}H_{24}O_2S$ 304.1497).

4.5. 3,17β-Bis(benzyloxy)-11β-methylthioestra-1,3,5(10)-triene (11)

Sodium powder (50% dispersion in oil, 0.5 g, 10.8 mmol) was washed free of oil and then suspended in dry THF (15 mL). Dimethyl disulfide (0.6 mL, 6.6 mmol) was added dropwise, and after 1 h the solvent was evaporated. Compound **9** (0.2 g, 0.36 mmol) dissolved in dry DMF (1 mL) was added to the residue, and the reaction mixture was stirred at 60 °C for 18 h. The reaction was partitioned between water and Et_2O, and the organic layer was washed with water, dried, and evaporated to give a residue from which **11** (0.1 g, 56% yield) was obtained by chromatography (9.7:0.3, hexane–EtOAc) as a glass. ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3H, 18-H), 2.06 (s, 3H, CH_3S), 3.48 (t, $J = 8$ Hz, 1H, 17-H), 3.60 (m, 1H, 11-H), 4.55, 4.99 (2s, 2×2H, PhCH_2), 6.7 (d, $J = 2.6$ Hz, 1H, 4-H), 6.8 (dd, $J = 2.6$ and 8.6 Hz, 1H, 2-H), 7.1 (d, $J = 8.6$ Hz, 1H, 1-H), 7.34 (m, 10H, *Ph*CH_2). EIMS m/z 498.2594 (calcd for $C_{33}H_{38}O_2S$ 498.2592).

4.6. 11β-Methylthioestra-1,3,5(10)-triene-3,17β-diol (5)

Debenzylation of **11** under the same conditions detailed for the conversion of **10** into **12** gave **5** (63% yield) as a glass. ^{1}H NMR (CD_3OD) δ 1.11 (s, 3H, 18-H), 2.02 (s, 3H, CH_3S), 3.64 (m, 2H, 11-H, 17-H), 6.44 (d, $J = 2.6$ Hz, 1H, 4-H), 6.55 (dd, $J = 2.6$, 8.5 Hz, 1H, 2-H), 7.04 (d, $J = 8.5$ Hz, 1H, 1-H). EIMS m/z 318.1653 (calcd for $C_{19}H_{26}O_2S$ 318.1653).

4.7. 3,17β-Bis(benzyloxy)-11α-methylthiomethylestra-1,3,5(10)-triene-11β-ol (14)

Metalation of dimethylsulfide was accomplished as reported.[9] To a solution of *n*-BuLi (2.2 mL, 2.6 mmol, 1.18 M in hexane), cooled at −5 °C, TMEDA (0.5 mL, 3.3 mmol), and dimethylsulfide (0.3 mL, 4 mmol) were added, under nitrogen. The reaction mixture was stirred at room temperature for 18 h, and then **13** (0.2 g, 0.4 mmol) was added. After 2 h the mixture was partitioned between Et_2O and 10% aq ammonium chloride. The organic layer was dried, filtered, and evaporated to give a residue from which **14** (180 mg, 85% yield) was obtained by chromatography (9:1, hexane–EtOAc) as glass. ^{1}H NMR ($CDCl_3$) δ 1.08 (s, 3H, 18-H), 2.19 (s, 3H, CH_3S), 2.77–2.84, 3.22–3.28 (ABq, 2H, CH_2S), 3.42 (t, $J = 8$ Hz, 1H, 17-H), 4.52, 5.0 (2s, 2×2H, PhCH_2), 6.74 (m, 2H, 2-H, 4-H), 7.32 (m, 10H, *Ph*CH_2), 7.7 (d, $J = 8.5$ Hz, 1H, 1-H). EIMS m/z 528.2700 (calcd for $C_{34}H_{40}O_3S$ 528.2698).

4.8. 3,17β-Bis(benzyloxy)-11β-methylthiomethylestra-1,3,5(10)-triene (15)

To a cooled (0 °C) solution of **14** (180 mg, 0.34 mmol) and borontrifluoride etherate (0.25 mL, 2 mmol) in methylene chloride (10 mL), triethylsilane (1 mL, 7 mmol) was added dropwise and the mixture was stirred at 0 °C for 1 h. The solution was partitioned between CH_2Cl_2 and 10% aq sodium carbonate. The organic layer was washed with ice/water, dried, and evaporated to give a residue from which **15** (120 mg, 69% yield) was obtained by chromatography (7:3, CH_2Cl_2–hexane) as a crystalline solid. Mp 95–97 °C. ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 3H, 18-H), 2.05 (s, 3H, CH_3S), 3.5 (m, $J = 8$ Hz, 1H, 17-H), 4.52, 4.58, 4.6, 4.66 (ABq, 2H, PhCH_2), 5.02 (s, 2H, PhCH_2), 6.69 (d, $J = 2.6$ Hz, 1H, 4-H), 6.8 (dd, $J = 2.6$, 8.5 Hz, 1H, 2-H), 7.1 (d, $J = 8.5$ Hz, 1H, 1-H), 7.36 (m, 10H, *Ph*CH_2). EIMS m/z 512.2749 (calcd for $C_{34}H_{40}O_2S$ 512.2749).

4.9. 11β-Methylthiomethylestra-1,3,5(10)-triene-3,17β-diol (6)

Debenzylation of **15** under the same conditions detailed for the conversion of **10** into **12** gave **6** (62% yield) as a glass. ^{1}H NMR (CD_3OD) δ 0.92 (s, 3H, 18-H), 2.01 (s, 3H, CH_3S), 3.64 (m, 1H, 17-H), 6.47 (d, $J = 2.5$ Hz, 1H, 4-H), 6.57 (dd, $J = 2.5$, 8.5 Hz, 1H, 2-H), 7.0 (d, $J = 8.5$ Hz, 1H, 1-H). EIMS m/z 332.1811 (calcd for $C_{20}H_{28}O_2S$ 332.1810).

4.10. 3,17β-Bis(benzyloxy)-11β-hydroxymethylestra-1,3,5(10)-triene (17)

A solution of **16** (0.5 g, 1 mmol), 4-methylmorpholine *N*-oxide (160 mg, 1.36 mmol), and osmium tetroxide (10 mg, 0.04 mmol) in acetone–water (4, 1 mL) was allowed to react until **16** was consumed (about 18 h). The reaction mixture was then partitioned between EtOAc and 10% aq sodium thiosulfate. The organic layer was washed with brine, dried, and evaporated to give a residue from which diol 3,17β-bis(benzyloxy)-11β-(1,2-dihydroxyethyl)estra-1,3,5(10)-triene (0.4 g, 78% yield) was obtained by chromatography (1:1, hexane–EtOAc) as a glass. ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 3H, 18-H), 4.58, 5.02 (2s, 2×2H, PhCH_2), 6.67 (d, $J = 2.6$ Hz, 1H, 4-H), 6.75 (dd, $J = 2.6$, 8.5 Hz, 1H, 2-H), 7.08 (d, $J = 8.5$ Hz, 1H, 1-H), 7.34 (m, 10H, *Ph*CH_2). EIMS m/z 512.2929 (calcd for $C_{34}H_{40}O_4$ 512.2927).

The above diol was dissolved in methylene chloride and added to a vigorously stirred suspension of silica gel (1.5 g) in CH_2Cl_2. A aqueous solution of sodium periodate (1.5 mL, 1.0 mmol, 0.65 M) was added dropwise, and after 30 min stirring at room temperature the organic layer was evaporated.[17] The residue was dissolved in methanol (20 mL) containing $NaBH_4$ (110 mg, 3 mmol), and the reaction mixture was allowed to equilibrate at room temperature for 5 h. A few drops of 10% aq HCl were added to destroy the excess of $NaBH_4$ and most of solvent was evaporated. The residue was partitioned between water and CH_2Cl_2. The organic layer was washed with $NaHCO_3$, dried, and evaporated to give almost pure **17** (210 mg, 56% yield) as a glass. ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H, 18-H), 3.44–3.78 (m, 4H, CH_2OH, 17-H), 4.58, 5.01 (2s, 2×2H, PhCH_2), 6.68–6.85 (m, 2H, 2-H, 4-H), 7.35 (m, 11H, 1-H, *Ph*CH_2). EIMS m/z 482.2823 (calcd for $C_{33}H_{38}O_3$ 482.2821).

4.11. 3,17β-Bis(benzyloxy)-11β-methanesulfonylmethylestra-1,3,5(10)-triene (19)

The alcohol **17** was mesylated as detailed for the conversion of **8** into **9** to give **19** (100% yield) as a glass. ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H, 18-H), 2.82 (s, 3H, CH_3–SO_3), 3.49 (q, $J = 8$ Hz, 1H, 17-H), 4.57, 5.02 (2s, 2×2H, PhCH_2), 4.09–4.27 (m, 2H, SO_2–OCH_2), 6.67 (d, $J = 2.6$ Hz, 1H, 4-H), 6.8 (dd, $J = 2.6$, 8.5 Hz, 1H, 2-H), 7.17 (d, $J = 8.5$ Hz, 1H, 1-H), 7.37 (m, 10H, *Ph*CH_2). EIMS m/z 560.2598 (calcd for $C_{34}H_{40}O_5S$ 560.2596).

4.12. 3,17β-Bis(benzyloxy)-11β-benzythiomethylestra-1,3,5(10)-triene (22)

The mesylate **19** was converted to the sulfide **22** (71% yield, glass) under the same conditions adopted for the conversion of **9** into **10**. ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 3H, 18-H), 3.43 (t, $J = 8$ Hz, 1H, 17-H), 3.62 (s, 2H, PhCH_2S), 4.48, 4.54, 4.57, 4.63 (ABq, 2H, PhCH_2), 4.98 (s, 2H, PhCH_2), 6.64–6.8 (m, 3H, 1-H, 2-H, 4-H), 7.3 (m, 15H, *Ph*CH_2). EIMS m/z 588.3063 (calcd for $C_{40}H_{44}O_2S$ 588.3062).

4.13. 11β-Benzylthiomethylestra-1,3,5(10)-triene-3,17β-diol (24)

Debenzylation of **22** under the same conditions detailed for the conversion of **10** into **12** gave **24** (61% yield) as a crystalline solid. Mp 98–100 °C. ^{1}H NMR (CD_3OD) δ

0.81 (s, 3H, 18-H), 3.62–3.72, (m, 3H, 17-H, PhCH_2S), 6.49 (m, 2H, 2-H, 4-H), 6.73 (d, $J = 9$ Hz, 1H, 1-H). EIMS m/z 422.2281 (calcd for $C_{27}H_{34}O_2S$ 422.2279).

4.14. 11β-Mercaptomethylestra-1,3,5(10)-triene-3,17β-diol (2)

S-Debenzylation of **24** under the same conditions detailed for the conversion of **12** into **1** gave **2** (52% yield) as a glass after chromatography (2:1, hexane–EtOAc). ^{1}H NMR (CD_3OD) δ 0.9 (s, 3H, 18-H), 3.65, (m, 1H, 17-H), 6.47 (d, $J = 2.6$ Hz, 1H, 4-H), 6.58 (dd, $J = 2.6$, 8.4 Hz, 1H, 2-H), 6.97 (d, $J = 8.5$ Hz, 1H, 1-H). EIMS m/z 318.1654 (calcd for $C_{19}H_{26}O_2S$ 318.1653).

4.15. 3,17β-Bis(benzyloxy)-11β-methylthioethylestra-1,3,5(10)-triene (21)

The mesylate **20** was converted to the sulfide **21** (60% yield, glass) under the same conditions adopted for the conversion of **9** into **11**. ^{1}H NMR ($CDCl_3$) δ 1.0 (s, 3H, 18H), 2.01 (s, 3H, CH_3S), 3.46 (q, $J = 8$ Hz, 1H, 17-H), 4.57, 5.01 (2s, 2×2H, PhCH_2), 6.67 (d, $J = 2.6$ Hz, 1H, 4-H), 6.77 (dd, $J = 2.6$, 8.5 Hz, 1H, 2-H), 7.1 (d, $J = 8.5$ Hz, 1H, 1-H), 7.35 (m, 10H, *Ph*CH_2). EIMS m/z 574.2906 (calcd for $C_{35}H_{42}O_2S$ 574.2905).

4.16. 11β-Methylthioethylestra-1,3,5(10)-triene-3,17β-diol (7)

Debenzylation of **21** under the same conditions detailed for the conversion of **10** into **12** gave **7** (63% yield) as a glass. ^{1}H NMR (CD_3OD) δ 0.9 (s, 3H, 18-H), 1.97 (s, 3H, CH_3S), 3.63, (m, 1H, 17-H), 6.48 (d, $J = 2.6$ Hz, 1H, 4-H), 6.57 (dd, $J = 2.6$, 8.4 Hz, 1H, 2-H), 7.01 (d, $J = 8.4$ Hz, 1H, 1-H). EIMS m/z 346.1968 (calcd for $C_{21}H_{30}O_2S$ 346.1966).

4.17. 3,17β-Bis(benzyloxy)-11β-benzylthioethylestra-1,3,5(10)-triene (23)

The mesylate **20** was converted to the sulfide **23** (71% yield, glass) under the same conditions adopted for the conversion of **9** into **10**. ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, 18-H), 3.45 (t, $J = 8$ Hz, 1H, 17-H), 3.64 (s, 2H, PhCH_2S), 4.5, 4.56, 4.59, 4.65 (ABq, 2H, PhCH_2), 5.0 (s, 2H, PhCH_2), 6.7–7.0 (m, 3H, 1-H, 2-H, 4-H), 7.51 (m, 15H, *Ph*CH_2). EIMS m/z 602.3220 (calcd for $C_{41}H_{46}O_2S$ 602.3218).

4.18. 11β-Benzylthioethylestra-1,3,5(10)-triene-3,17β-diol (25)

Debenzylation of **23** under the same conditions detailed for the conversion of **10** into **12** gave **25** (70% yield) as a glass. ^{1}H NMR (CD_3OD) δ 0.8 (s, 3H, 18-H), 3.6–3.7, (m, 3H, 17-H, PhCH_2S), 6.48 (d, $J = 2.4$ Hz, 1H, 4-H), 6.59 (dd, $J = 2.4$, 8.4 Hz, 1H, 2-H), 6.97 (d, $J = 8.5$, 1H, 1-H). EIMS m/z 422.2278 (calcd for $C_{27}H_{34}O_2S$ 422.2279).

4.19. 11β-Mercaptoethylestra-1,3,5(10)-triene-3,17β-diol (3)

S-Debenzylation of **25** under the same conditions detailed for the conversion of **12** into **1** gave **3** (60% yield) as a glass after chromatography (2:1, hexane–EtOAc). ^{1}H NMR (CD_3OD) δ 0.87 (s, 3H, 18-H), 3.62, (m, 1H, 17-H), 6.46 (d, $J = 2.5$ Hz, 1H, 4-H), 6.57 (dd, $J = 2.5$, 8.4 Hz, 1H, 2-H), 6.98 (d, $J = 8.5$ Hz, 1H, 1-H). EIMS m/z 332.1811 (calcd for $C_{20}H_{28}O_2S$ 332.1810).

4.20. 3,17β-Bis(methoxy)estra-1,3,5(10)-triene-6-ol (27)

To a cooled (−15 °C) mixture of **26** (2.5 g, 8.3 mmol), *t*-BuOK (1.87 g, 16.6 mmol), and diisopropylamine (210 mg, 2.07 mmol, 0.29 mL) in dry THF (20 mL), *n*-BuLi (9.34 mL, 1.6 M in hexane) were added over a 10-min period, and the resulting dark-red solution further stirred for 10 min. Trimethyl borate (2.06 g, 20 mmol, 2.2 mL) was added, and to the resulting milky solution was finally added 35% aq H_2O_2 (10 mL). After 1 h stirring at room temperature, the reaction mixture was partitioned between toluene and diluted with 10% aq HCl. The organic layer was washed with water, dried with Na_2CO_3, and evaporated to give a residue from which **27** (2.1 g, 80% yield) was obtained by chromatography (2:1, hexane–EtOAc) as a single stereoisomer as an oil, which solidified upon standing. Mp 92–95 °C. ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H, 18-H), 3.36 (s, 3H, 17-OCH_3), 3.8 (s, 3H, 3-OCH_3), 4.8 (m, 1H, 6-H), 6.7 (dd, $J = 2.9$, 8.8 Hz, 1H, 2-H), 7.1 (d, $J = 2.9$ Hz, 1H, 4-H), 7.2 (d, $J = 8.8$ Hz, 1H, 1-H). EIMS m/z 316.2040 (calcd for $C_{20}H_{28}O_3$ 316.2038).

4.21. 3,17β-Bis(methoxy)estra-1,3,5(10)-triene-6-one (28)

The alcohol **27** (1 g, 3 mmol) was added to a suspension of PCC (1.3 g, 6 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature until starting material was consumed (about 1 h, TLC monitoring). Ethyl ether (30 mL) was added, the mixture was filtered through Florisil, and the filtrate was evaporated to give a residue from which pure **28** (1 g, 100% yield) was obtained as a white solid after trituration with ether. Mp 133–135 °C. ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H, 18-H), 2.7 (dd, $J = 3.4$, 16.6 Hz, 1H, 8-H), 3.32 (t, $J = 8$ Hz, 1H, 17-H), 3.36 (s, 3H, 17-OCH_3), 3.83 (s, 3H, 3-OCH_3), 7.08 (dd, $J = 2.9$, 8.8 Hz, 1H, 2-H), 7.33 (d, $J = 8.8$ Hz, 1H, 1-H), 7.5 (d, $J = 2.9$ Hz, 1H, 4-H). EIMS m/z 314.1885 (calcd for $C_{20}H_{26}O_3$ 314.1882).

4.22. 7α-Bromo-3,17β-bis(methoxy)estra-1,3,5(10)-triene-6-one (29)

To a cooled (−5°C) solution of **28** (2 g, 6.3 mmol) in CH_2Cl_2 (10 mL) bromine (900 mg, 5.6 mmol, 29 mL, 1 M

in CCl_4) was added dropwise, and the mixture was stirred until the starting material was consumed (TLC monitoring). The reaction mixture was then partitioned between water and 10% aq sodium bicarbonate. The organic layer was washed with 10% aq sodium thiosulfate, dried, and evaporated to give a residue from which pure **29** (2.5 g, 100% yield) was obtained as a crystalline solid after trituration with ether. Mp 147–148 °C. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, 3H, 18-H), 3.35 (s, 4H, 17-H, 17-OCH_3), 3.82 (s, 3H, 3-OCH_3), 4.48 (d, 1H, $J = 2$ Hz, 7-H), 7.1 (dd, $J = 2.9$, 8.8 Hz, 1H, 2-H), 7.33 (d, $J = 8.8$ Hz, 1H, 1-H), 7.5 (d, $J = 2.9$ Hz, 1H, 4-H). EIMS m/z 392.0988 (calcd for $C_{20}H_{25}BrO_3$ 392.0987).

4.23. 7α-Benzylthio-3,17β-bis(methoxy)estra-1,3,5(10)-triene-6-one (30)

Conditions A. Compound **29** (100 mg, 0.25 mmol) was added to a mixture of *t*-BuOK (42 mg, 0.37 mmol) and benzyl mercaptan (0.06 mL, 0.5 mmol) in DMF (2 mL). The mixture was heated at 50 °C for 20 min and then stirred at room temperature for 6 h. The reaction mixture was partitioned between water and CH_2Cl_2, and the organic layer was dried and evaporated to give a residue from which pure **30** (96 mg, 90% yield) was obtained as a crystalline solid after trituration with ether/hexane. *Conditions B.* To a mixture of **29** (150 mg, 0.38 mmol), 20% aq NaOH (3.8 mL, 1.9 mmol), and tetrabutylammonium hydrogensulfate (260 mg, 0.76 mmol) in benzene (4 mL) was added benzyl mercaptan (0.08 mL, 0.6 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was partitioned between water and Et_2O, and the organic layer was dried and evaporated to give a residue from which pure **30** (150 mg, 90% yield) was obtained as a crystalline solid after trituration with hexane. Mp 163–165 °C. ^{1}H NMR ($CDCl_3$) δ 0.71 (s, 3H, 18-H), 3.24 (d, $J = 2.9$ Hz, 1H, 7-H), 3.32 (m, 4H, 17-H, 17-OCH_3), 3.65–3.71, 3.73–3.8 (ABq, 2H, PhCH_2S), 3.87 (s, 3H, 3-OCH_3), 7.1 (dd, $J = 2.4$, 8.5 Hz, 1H, 2-H), 7.35 (m, 6H, 1-H, *Ph*CH_2S), 7.68 (d, $J = 2.4$ Hz, 1H, 4-H). EIMS m/z 436.6074 (calcd for $C_{27}H_{32}O_3S$ 436.6072).

4.24. 7α-Benzylthio-3,17β-bis(methoxy)estra-1,3,5(10)-triene (31)

Deoxygenation of **30** under the same conditions detailed for the conversion of **14** into **15** gave **31** (82% yield) as a crystalline solid after trituration with ether. Mp 148–152 °C. ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 3H, 18-H), 2.95–3.03, 3.13–3.18 (ABXq, 2H, 6-H), 3.35 (m, 4H, 17-H and 17-OCH_3), 3.78 (s, 3H, 3-OCH_3), 6.61 (d, $J = 2.4$ Hz, 1H, 4-H), 6.73 (dd, $J = 2.4$, 8.5 Hz, 1H, 2-H), 7.2 (m, 6H, 1-H, *Ph*CH_2S). EIMS m/z 422.2281 (calcd for $C_{27}H_{34}O_2S$ 422.2279).

4.25. 7α-Mercapto-3,17β-bis(methoxy)estra-1,3,5(10)-triene (32)

S-Debenzylation of **31** under the same conditions detailed for the conversion of **12** into **1** gave **32** (73% yield) as a glass. ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H, 18-H), 3.36 (s, 3H, 17-OCH_3), 3.76 (s, 3H, 3-OCH_3), 6.58 (d, $J = 2.4$ Hz, 1H, 4-H), 6.73 (dd, $J = 2.4$, 8.8 Hz, 1H, 2-H), 7.22 (d, $J = 8.8$ Hz, 1H, 1-H). EIMS m/z 332.1811 (calcd for $C_{20}H_{28}O_2S$ 332.1810).

4.26. 7α-Mercapto-3,17β-dihidroxyestra-1,3,5(10)-triene (4)

Aluminum tribromide (160 mg, 0.6 mmol) was generated as follows: bromine (1 mL, 1 mmol) was added dropwise to a suspension of aluminum scales (18 mg, 0.6 mmol) in CH_2Cl_2 (10 mL); the mixture was refluxed until the red color was discharged and a white solid was formed. To the above suspension, stirred at 0 °C, a solution of **32** (50 mg, 0.12 mmol) and EtSH (2 mL) in CH_2Cl_2 (3 mL) was added, and the reaction mixture was stirred at room temperature for 6 h. The mixture was partitioned between ice/water and EtOAc. The organic layer was washed with brine, dried, and evaporated to give a residue from which **4** (83% yield) was obtained as a crystalline solid after trituration with ether/CH_2Cl_2. Mp 162–163 °C. ^{1}H NMR (CD_3OD) δ 0.66 (s, 3H, 18-H), 3.56 (t, 1H, 17-H), 6.35 (d, $J = 2.4$ Hz, 1H, 4-H), 6.46 (dd, $J = 2.4$, 8.8 Hz, 1H, 2-H), 6.99 (d, $J = 8.8$ Hz, 1H, 1-H). EIMS m/z 304.1499 (calcd for $C_{18}H_{24}O_2S$ 304.1497).

4.27. Estrogen receptor binding affinity determination

Relative binding affinities were determined by competitive radiometric binding assay using 10 nM [^{3}H]estradiol as tracer as previously described,[18] using uterine cytosol diluted to approximatively 1.5 nM of receptor. 18 incubations were done at 0 and 25 °C for 18–24 h.

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