Pergamon

0960-0760(95)00014-3

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 6, pp. 547–565, 1995

0960-0760/95 $9.50 + 0.00

Synthesis and Biological Activities of Thioether Derivatives Related to the Antiestrogens Tamoxifen and ICI 164384

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The catalyzed coupling reaction of activated alcohol and mercaptan was used for the short and efficient synthesis of 14 thioether compounds. Two types of side chains, the methyl butyl alkylamide related to the pure steroidal antiestrogen ICI 164384 and the dimethylamino ethyloxy phenyl related to the clinically used nonsteroidal antiestrogen tamoxifen, were introduced by a thioether link on two types of nuclei (triphenylethane or estradiol). The new thioether derivatives were tested to assess their relative binding affinity for the estrogen receptor and their estrogenic or antiestrogenic activity in the ZR-75-1 (ER^+) cell line. The results indicate that of the three types of compounds studied, only the nonsteroidal derivatives with an alkylamide side chain possess antiestrogenic activity. In the steroidal series, displacement of the alkylamide side chain from the 7 to the 6 position produced compounds with chemical characteristics similar to ICI 164384 or EM-139 but without antiestrogenic activity. In the nonsteroidal series of compounds with an aryl side chain, compounds with estrogenic activity were obtained. One compound, a nonsteroidal derivative with a methyl butyl alkylamide side chain 20, possesses a relative binding affinity for the estrogen receptor identical to EM-139 (1.1 and 1.2%, respectively) and a relatively good antiestrogenic activity that is 10-fold lower than EM-139 (IC_{50} values of 250 and 25 nM, respectively). This nonsteroidal thioether with an alkylamide side chain is free of estrogenic activity.

J. Steroid Biochem. Molec. Biol., Vol. 52, No. 6, pp. 547–565, 1995

INTRODUCTION

Breast cancer is the most frequent cancer in women and the leading cause of cancer death. In fact, one in eight North American women suffers from breast cancer during her lifetime, and 46,000 women were expected to die of this disease in 1993 [1]. Because estrogens are the best known stimulator of breast cancer development and growth, antiestrogens are considered good candidates for the therapy of this disease [2, 3]. However, the nonsteroidal antiestrogen tamoxifen (**1**) (Fig. 1), currently used for the treatment of breast cancer in women, possesses mixed estrogenic and antiestrogenic activation and cannot be considered a pure antiestrogen [4–7]. Analogues of tamoxifen (**1**) or its direct metabolite hydroxy-tamoxifen (**2**) [8] have been synthesized to decrease agonistic activity and improve antagonistic activity. The recently developed 4-iodotamoxifen (**3**) and pyrrolidino-4-iodotamoxifen (**4**) [9] show improved antagonistic activity with lower agonistic activity than tamoxifen, but these compounds still possess residual agonistic activity. Jones *et al.* [10] synthesized trioxifen (**5**) and reported it to be a better antiestrogen than tamoxifen in that it (**1**) has fewer agonistic effects. LY-117018 (**6**) [11] and LY-139481 (**7**) [12], developed later, have some advantages over trioxifen. More recently, the same group developed benzo[a] fluorene derivative (**8**) [13], although this compound is comparable to LY-117018 (**6**) and LY-139481 (**7**). The most effective compounds of this series (LY-117018 and LY-139481) are rapidly conjugated, metabolized, and excreted, thus explaining their low potency as antitumour agents in animals [8, 14–16].

During the last decade, a new generation of steroidal compounds has been described as pure antiestrogens [17–22]. These compounds, represented by ICI 164 384 (**9**) [19] and EM-139 (**10**) [21], contain a long methyl butyl alkylamide side chain at the 7α-position of the estradiol nucleus. Other groups introduced var-

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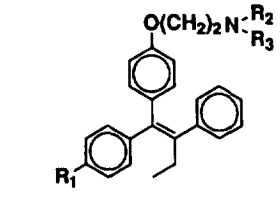
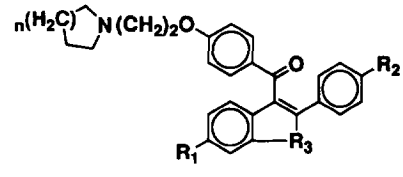
Received 6 Jun. 1994; accepted 13 Jan. 1995.

ious side chains at different positions of the estradiol nucleus to improve biological activity. Thus, Nedelec *et al.* [23, 24] introduced an aryl side chain like that of tamoxifen at the 7- or 11-position of the estradiol nucleus. The most effective compounds of this series, RU 45144 (7α-side chain) [23] and RU 39411 (**11**) (11β-side chain) [24], are potent antiestrogenic and antiproliferative molecules; but these compounds, like tamoxifen, also display partial estrogenic activity. Poirier *et al.* introduced methyl butyl alkylamide side chains at the 15- or 17α-position of estradiol [25, 26] and a similar alkynylamide side chain at the 17α-position of estradiol [27]. These compounds, however, did not improve the antiestrogenic activity (unpublished data and references [25, 27]). Claussner *et al.* [28] introduced a methyl i-propyl alkylamide side chain at the 11β-position of estradiol (RU 51625 (**12**)). These products were completely devoid of uterotrophic activity when administered subscutaneously in mice, but they exhibited a slight agonistic effect when administered orally. Wakeling *et al.* [29] described another pure steroidal antiestrogen, ICI 182780 (**13**). This compound, which contains a long alkysulfoxide side chain in position-7α of estradiol, was 10-fold more potent than ICI 164384 [29]. Nonsteroidal antiestrogens related to indolic derivatives **14** [30] were also described recently. These compounds, derived from the indolic nucleus, cannot be considered pure antiestrogens because they show agonistic activity [31]. At this time, estradiol derivatives with an alkylamide side chain in the 7α or 11β position as well as an alkylsulfoxide side chain in the 7α position appear to be promising antiestrogens. On the other hand, these interesting pure steroidal antiestrogens have the disadvantage of their long chemical synthesis.

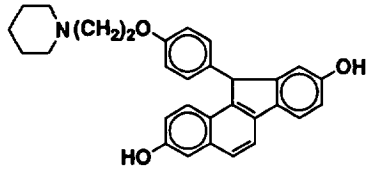
To obtain more easily compounds with characteristics similar to those of ICI 164384 (**9**), EM-139 (**10**), and tamoxifen (**1**) and in continuation of our recently reported synthesis of diaryl thioether derivatives [32], we synthesized a series of nonsteroidal and steroidal derivatives (Fig. 2). All compounds were synthesized according to a short sequence using the formation of a thioether as a key step [32]. Compounds of the first series (nucleus A) contain the important methyl butyl amide side chain added to a diaryl butane substrate. Compounds of second series (nucleus B) contain a thioaryl side chain similar to tamoxifen, and those in the last series (nucleus C) comprise a steroid nucleus with an alkylamide side chain at the C6 position. These molecules possess a 3-hydroxy group (pseudo-3-hydroxyl group for nonsteroidal nucleus) and an alkylamide or aryl amine side chain, two parts of molecule that are important, respectively, for their binding and antiestrogenic activity. In this work, we report the short, efficient synthesis of 14 new compounds, their relative binding affinity on the estrogen receptor, and their *in vitro* estrogenic or antiestrogenic activity in the



1 Tamoxifen (R_1=H, R_2=R_3=CH_3)
2 OH-Tamoxifen (R_1=OH, R_2=R_3=CH_3)
3 4-Iodotamoxifen (R_1=I, R_2=R_3=CH_3)
4 Pyrrolidino-4-iodotamoxifen (R_1=I, R_2,R_3= -$(CH_2)_4$-)



5 Trioxifen (R_1=H, R_2=OCH_3, R_3=-$(CH_2)_2$-, n=1)
6 LY-117018 (R_1=R_2=OH, R_3=S, n=1)
7 LY-139481 (R_1=R_2=OH, R_3=S, n=2)



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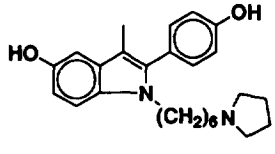
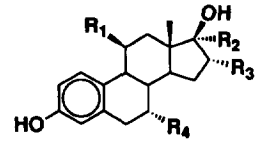
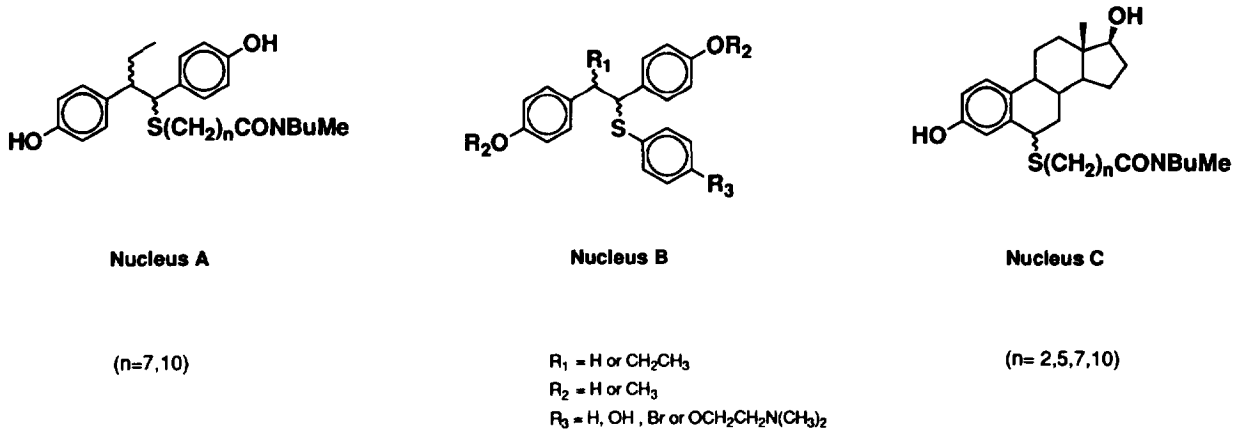


14 ZK 119010



9 ICI 164384 (R_1=R_2=R_3=H, R_4=$(CH_2)_{10}CONBuMe$)
10 EM-139 (R_1=R_2=H, R_3=Cl, R_4=$(CH_2)_{10}CONBuMe$)
11 RU 39411 (R_1=$PhO(CH_2)_2N(CH_3)_2$, R_2=R_3=R_4=H)
12 RU 51625 (R_1=$(CH_2)_{10}CONMeiPr$, R_2=R_3=R_4=H)
13 ICI 182780 (R_1=R_2=R_3=H, R_4=$(CH_2)_9SO(CH_2)_3CF_2CF_3$)

Fig. 1. Molecular structures of several known antiestrogens.



Nucleus A (n=7,10)

Nucleus B R_1 = H or CH_2CH_3; R_2 = H or CH_3; R_3 = H, OH, Br or $OCH_2CH_2N(CH_3)_2$

Nucleus C (n= 2,5,7,10)

Fig. 2. Molecular structures of synthesized thioether derivatives. Nonsteroidal thioethers with an alkylamide side chain (Nucleus A); nonsteroidal thioethers with an aryl side chain (Nucleus B); and steroidal thioethers with an alkylamide side chain (Nucleus C).

estrogen-sensitive ZR-75-1 human breast cancer cell line.

EXPERIMENTAL

Chemical Synthesis

General procedure

Thin-layer chromatography was performed on 0.20 mm silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany); 230–400 mesh ASTM silica gel 60 (E. Merck) was used for flash column chromatography. When thioethers were submitted to biological tests, last-step chromatography was performed with freshly distilled or high-performance liquid chromatography (HPLC)-grade solvents. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1310 or 1600 (series FTIR) spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC/F 300 spectrometer using TMS as internal standard. For ^{13}C NMR spectra, only the important carbons were assigned (between [ ]); duplication of signals attributed to the amide group are given in parentheses. Mass spectra were recorded with a V.G. Micromass 16 F spectrometer; the exact mass (EIMS or FABMS, thioglycerol matrix) was provided by the Centre Régional de Spectrométrie de Masse (Université de Montréal, Montréal, Canada).

Synthesis of nonsteroidal thioethers with an alkylamide chain: 19–21 (Fig. 3)

Synthesis of 1,2-di(4'-methoxy phenyl)-1-butanone (16)

To a solution of dry THF (30 ml) and dry diisopropylamine (57 mmol) at 0°C, under an argon atmosphere, dropwise butyllithium (22 ml of 2.5 M solution in hexane, 55 mmol) was added over 15 min and allowed to react for 1 h. Then the solution of desoxyanisoin **15** (79 mmol) in dry THF (100 ml) was added at 0°C and the mixture was stirred. After 1 h, ethyl bromide was added and the cooling bath removed. The mixture was refluxed overnight. The reaction was cooled and neutralized by the addition of HCl (1N). The mixture was extracted with diethyl ether, washed with water, and dried over $MgSO_4$. After evaporation of the solvent, purification of the crude compound was done by column chromatography with hexane-EtOAc, 90:10 as eluent to produce a colorless oil (77% yield); IR ν (neat) 1670 (C=O, conjugated ketone); ^1H NMR δ ($CDCl_3$, 300 MHz), 0.90 (t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$), 1.83 and 2.18 (2m, 2H, 2 × H of $\underline{CH_2}CH_3$, isomers R:S/50:50), 3.69 and 3.74 (2s, 6H, 2 × CH_3O), 4.38 (t, J = 7.3 Hz, 1H, CHCO), 6.82 (d, J = 8.6 Hz, 2H, ArH m-to CO); 6.84 (d, J = 8.6 Hz, 2H, ArH m-to CH), 7.24 (d, J = 8.7 Hz, 2H, ArH o-to CH), 7.97 (d, J = 8.9 Hz, 2H, ArH o-to CO); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 12.1 [CH_3 of Et], 26.9 [CH_2 of Et], 53.9 [CH], 54.9, 55.1, 113.5 (2 ×), 114.0 (2 ×), 129.0 (2 ×), 129.8, 130.7 (2 ×), 131.9, 158.3, 163.0, 198.6 [C=O]; MS *m/e* 284 (M^+, 36), 149 (100), 135 (100), 121 (89), 107 (29); EIMS calculated for $C_{18}H_{20}O_3$(M^+) 284.1412 found 284.1376.

Synthesis of 1,2-di{4'-[(tetrahydro-2"H-pyran-2"yl)-oxy]phenyl}-1-butanone (17)

Di-methoxyphenyl butanone **16** (0.47 mmol) was demethylated by pyridine hydrochloride treatment according to the procedure of Buu-Hoi *et al.* [33]. Without purification, the crude diphenolic compound was dissolved in benzene (2.5 ml) and 3,4-dihydro-2H-pyran (0.5 ml). A catalytic amount of p-TSA (14 mg) was added, and the mixture was stirred at 0°C under argon for 20 h. The mixture was poured into a saturated $NaHCO_3$ solution, extracted with EtOAc, washed in water, and dried over $MgSO_4$. After evapor-

ation of the solvent, the crude compound was purified by column chromatography with hexane-EtOAc 90:10 as eluent to produce 88 mg (49% yield) of a white solid, mp 135–136°C; IR ν (KBr) 1663 (C=O, conjugated ketone); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.88 (t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$), 1.5–2.1 (m, 13H, 6 × CH_2 of THP and 0.5 $\underline{CH_2}CH_3$ (one isomer)), 2.15 (m, 1H, 0.5 × $\underline{CH_2}CH_3$ (one isomer)), 3.60 and 3.85 (2m, 4H, 2 × CH_2O of THP), 4.34 (t, J = 7.3 Hz, 1H, CHCO), 5.34 and 5.46 (2t, J = 3.0 Hz, 2H, 2 × CH of THP), 6.95 (d, J = 8.7 Hz, 2H, ArH m-to CO), 7.01 (d, J = 8.9 Hz, 2H, ArH m-to CH), 7.19 (d, J = 8.5 Hz, 2H, ArH o-to CH), 7.93 (d, J = 8.8 Hz, 2H, ArH o-to CO); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 12.2 [CH_3 of Et], 18.4, 18.8, 25.0, 25.1, 27.0 [CH_2 of Et], 30.0, 30.3, 54.2 [CH], 61.9 and 62.0 [CH_2O of THP], 95.9 and 96.4 [OCHO of THP], 115.8 (2 ×), 116.6 (2 ×), 129.1 (2 ×), 130.7 (3 ×), 132.9, 156.0, 160.6, 198.8 (C=O); MS *m/e* 424 (M^+, 0.2), 340 (7.4), 256 (67), 205 (42), 135 (100), 121 (100), 107 (63), 85 (100); FABMS calculated for $C_{26}H_{33}O_5$(M^+ + H) 425.2328 found 425.2363.

Synthesis of 1,2-di{[tetrahydro-2″H-pyran-2″yl)oxy]-phenyl}-1-butanol (18)

A mixture of ketone 17 (2.36 mmol), methanol (130 ml), and sodium borohydride (3 equiv.) was stirred at room temperature for 24 h. The reaction was quenched by the addition of water, and methanol was evaporated under reduced pressure. The resulting solid was filtered, washed with water, and dried under vacuum pump for 1 or 2 days to produce 0.82 g of a white solid (82% yield), mp 115–120°C; IR ν (KBr) 3422 (OH, alcohol): ^{1}NMR δ ($CDCl_3$, 300 MHz), 0.61 (t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$), 1.3–2.1 (m, 14H, 6 × CH_3 of THP and $\underline{CH_2}CH_3$), 2.65 (m, 1H, CHCHOH), 3.61 and 3.95 (2m, 4H, 2 × CH_2O of THP), 4.60 (d, J = 8.7 Hz, 1H, CHOH), 5.40 (m, 2H, 2 × CH of THP), 7.03 and 7.04 (2d, J = 8.6 Hz, 4H, ArH m-to CH and ArH m-to CHOH), 7.16 (d, J = 8.6 Hz, 2H, ArH o-to CH), 7.25 (d, J = 8.5 Hz, 2H, ArH o-to CHOH); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 12.0 (CH_3 of Et), 18.8, 18.9, 25.1 [CH_2 of Et], 25.2 (2 ×), 30.4 (2 ×), 55.6 [CH], 78.4 [(CHOH], 62.0 and 62.2 [CH_2O of THP], 96.4 and 96.5 [OCHO of THP], 116.2 (2 ×), 116.6 (2 ×), 128.1 (2 ×),

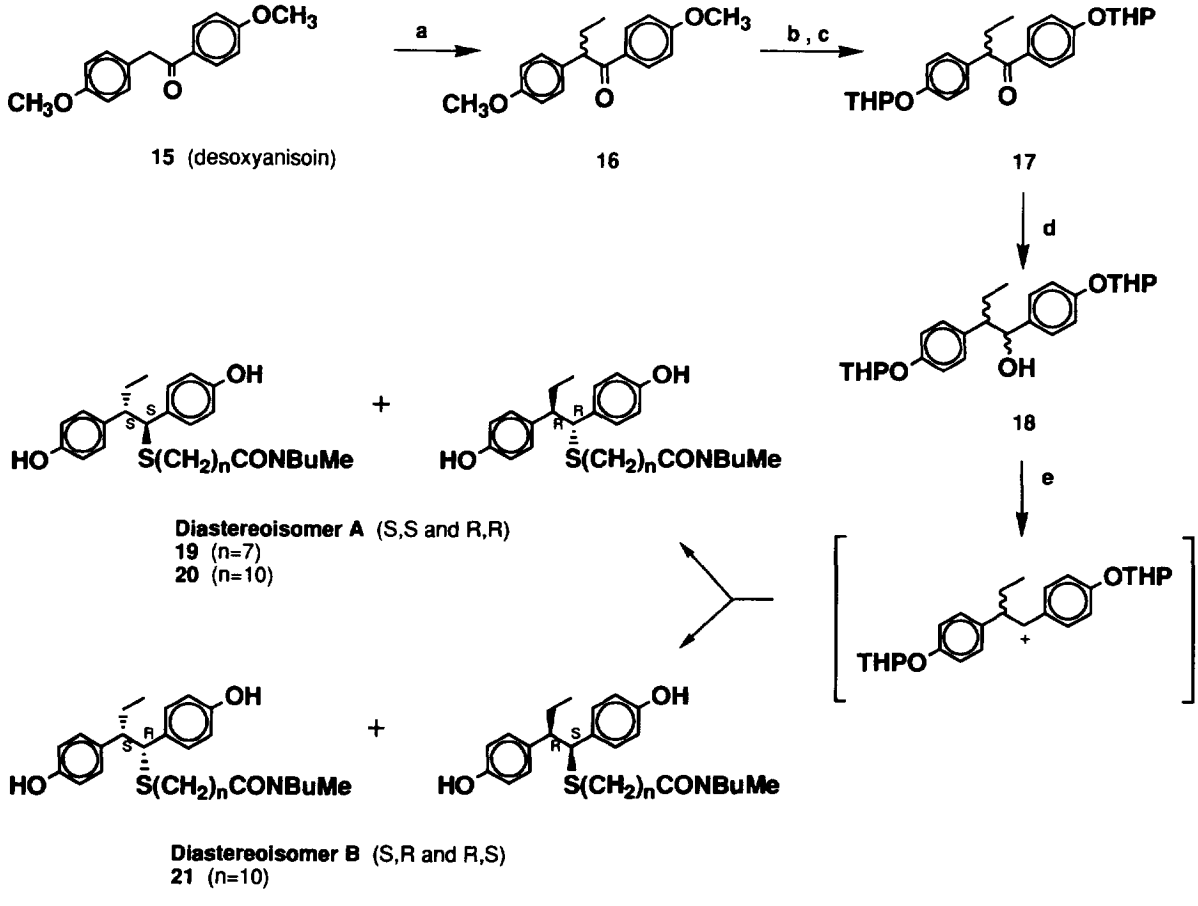


Fig. 3. Synthesis of nonsteroidal thioethers with an alkylamide side chain (19–21). The reagents are (a) 1. LDA, THF, 0°C; 2. EtBr, reflux; (b) pyr.HCl, reflux; (c) DHP, *p*-TSA, benzene; (d) $NaBH_4$, MeOH; (e) $HS(CH_2)_nCONBuMe$ (n = 7, 10), ZnI_2, $ClCH_2CH_2Cl$.

Table 1. Experimental conditions used for the synthesis of thioethers

Alcohol		Side chain		Zinc iodide	Solvent	Time	Thioether	
No.	mmol	No.*	mmol	(mmol)	(ml)	(h)	No.	Yield (%)
18	0.18	c	0.26	0.53	1	17	19	47†
18	3.08	d	3.85	4.59	60	24	20 + 21	75‡
23	0.75	g	0.90	1.13	10	18	24	91
23	0.75	h	0.90	1.13	10	24	25	16
23	0.75	e	0.90	1.13	10	48	26	95
22	3.88	e	4.65	5.81	30	48	27	64
23	0.42	f	0.63	0.42	2	17	28	93
18	2.35	f	3.52	2.82	30	10	29 + 30	89§
41	4.10	a	6.10	6.10	300	17	42	98
41	0.35	b	0.46	0.35	5	17	43	60
41	1.00	c	1.50	0.73	100	10	44	45
41	0.65	d	0.85	0.98	20	18	45	42

*Side chain used: (a) $HS(CH_2)_2COOCH_3$; (b) $HS(CH_2)_5CONBuMe$; (c) $HS(CH_2)_7CONBuMe$; (d) $HS(CH_2)_{10}CONBuMe$; (e) HS-Ph-OH; (f) HS-Ph-OTBDMS; (g) HS-Ph; (h) HS-Ph-Br.

†Only for major diastereoisomer A (less polar on silica gel).

‡In a proportion of 59:41 for **20** and **21**, respectively.

§In a proportion of 72:28 for **29** and **30**, respectively.

129.6 (2 ×), 134.1, 136.0, 156.1, 156.7; MS *m/e* 325 ($M^+ - (H_2O + DHP)$, 0.3), 240 (37), 136 (44), 123 (100), 107 (49), 85 (100); FABMS calculated for $C_{26}H_{34}NaO_5$ ($M^+ + Na$) 449.2304 found 449.2297.

Synthesis of racemic thioethers: ***19–21***

Zinc iodide was added to a solution of racemic alcohol **18** and mercapto side chain in dry 1,2-dichloroethane. The suspension was stirred at room temperature under an argon atmosphere for the appropriate time. The reaction was then quenched by the addition of water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated to dryness; the crude product was purified by column chromatography using a suitable mixture of hexane-EtOAc as eluent. Table 1 shows the conditions used for thioether formation.

*N-butyl, N-methyl 10,11-di(4'-hydroxy phenyl)-9-thiatridecanamide (**19**)*

Only one racemic mixture of enantiomers (diastereoisomer A, the major less polar on silica gel) was recovered. Colorless oil, IR *v* (neat) 3260 (OH, phenol), 1610 (C=O, amide); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.58 (t, J = 7.2 Hz, 3H, $CHCH_2\underline{CH_3}$), 0.93 and 0.95 (2t, J = 6.6 Hz, 3H, $CH_2CH_2\underline{CH_3}$), 1.0–1.4 (m, 11H, 5 × CH_2 and 0.5 × $CH\underline{CH_2}CH_3$), 1.5 (m, 5H, $\underline{CH_2}CH_2CONCH_2\underline{CH_2}$ and 0.5 × $CH\underline{CH_2}CH_3$), 1.9 and 2.1 (2m, 2H, $S\underline{CH_2}$), 2.28 (m, 2H, $\underline{CH_2}CO$), 2.72 (td, J_1 = 3.2 Hz and J_2 = 10.2 Hz, 1H, PhC<u>H</u>CHS), 2.96 and 2.98 (2s, 3H, CH_3NCO), 3.26 and 3.40 (2t, J = 6.7 Hz, 2H, CH_2NCO), 3.78 (d, J = 10.3 Hz, 1H, CHS), 6.83 (d, J = 7.4 Hz, 4H, ArH m-to CH and m-to CHSR), 7.05 (d, J = 8.4 Hz, 2H, ArH o-to CH), 7.15 (d, J = 8.4 Hz, 2H, ArH o-to CHSR); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 12.1 [CH_3 of Et], 13.9, 19.9 (20.0), 25.2 (25.6), 27.2 [CH_2 of Et], 27.9, 28.6 (2 ×), 29.2, 29.3 (30.5), 30.9, 33.1 (33.8), 33.8 (35.7) [CH_3N], 47.9 (50.1) [CH_2N], 53.2 [CHEt], 56.1 [CHS], 115.3 (2 ×), 115.5 (2 ×), 129.2 (2 ×), 129.5 (2 ×), 133.6, 134.3, 154.9, 155.3, 174.2 [CON]; MS *m/e*: 485 (M^+, 0.6), 350 (M^+ – HOPhCHEt, 100), 240 (52), 211 (60), 42 (28), 114 (57), 107 (54); FABMS calculated for $C_{29}H_{44}NO_3S$ ($M^+ + H$) 486.3042 found 486.3063.

*N-butyl, N-methyl 13,14-di(4'-hydroxy phenyl)-12-thiahexadecanamide (**20 or 21**)*

Diastereoisomer A, a racemic mixture of enantiomers less polar on silica gel: ***20.*** Colorless oil; IR *v* (neat) 3250 (OH, phenol), 1610 (C=O, amide); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.57 (t, J = 7.3 Hz, 3H, $CHCH_2\underline{CH_3}$), 0.91 and 0.95 (2t, J = 7.3 Hz, 3H, $CH_2CH_2\underline{CH_3}$), 1.0–1.4 (m, 17H, 8 × CH_2 and 0.5 × $CH\underline{CH_2}CH_3$), 1.6 (m, 5H, $\underline{CH_2}CH_2CONCH_2\underline{CH_2}$ and 0.5 × $CH\underline{CH_2}CH_3$), 2.0 and 2.1 (2m, 2H, $S\underline{CH_2}$), 2.34 (m, 2H, $\underline{CH_2}CO$), 2.71 (td, J_1 = 3.0 Hz and J_2 = 10.2 Hz, 1H, PhC<u>H</u>CHS), 2.95 and 2.99 (2s, 3H, CH_3NCO), 3.28 and 3.40 (2t, J = 2.5 Hz, 2H, CH_2NCO), 3.87 (d, J = 9.8 Hz, 1H, CHS), 6.81 and 6.84 (2d, J = 8.8 Hz, 4H, ArH m-to CH and m-to CHSR), 7.01 (d, J = 8.3 Hz, 2H, ArH o-to CH), 7.13 (d, J = 8.3 Hz, 2H, ArH o-to CHSR); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 12.1 [CH_3 of Et], 13.9, 19.9 (20.0), 25.0 (25.4), 27.2 [CH_2 of Et], 28.0 to 29.3 (7 ×), 29.4 (30.6), 30.8, 32.9 (33.6), 33.8 (35.6) [CH_3N], 47.9 (50.1) [CH_2N], 53.3 [CHEt], 55.7 [CHS], 115.1 (2 ×), 115.2 (×), 129.3 (2 ×), 129.7 (2 ×), 133.0, 134.2, 154.8, 155.5, 174.1 [CON]; MS *m/e* 392 (M^+ – HOPhCHEt, 17), 286 (40), 240 (32), 211 (37), 142 (53), 114 (100); FABMS calculated for $C_{32}H_{48}NO_3S$ ($M^+ - H$) 526.3355 found 526.3335.

Diastereoisomer B, a racemic mixture of enantiomers more polar on silica gel: ***21.*** Colorless oil; IR *v* (neat)

3250 (OH, phenol), 1605 (C=O, amide); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.70 (t, J = 7.3 Hz, 3H, $CHCH_2\underline{CH_3}$), 0.88 and 0.93 (2t, J = 7.4 Hz, 3H, $CH_2CH_2\underline{CH_3}$, 1.0–1.4 (m, 17H, 8 × CH_2 and 0.5 × $CH\underline{CH_2}CH_3$), 1.4–1.7 (m, 5H, $\underline{CH_2}CH_2CONCH_2\underline{CH_2}$ and 0.5 × $CH\underline{CH_2}CH_3$) 2.1 and 2.2 (2m, 2H, SCH_2), 2.29 (m, 2H, $\underline{CH_2}CO$), 2.8 (m, 1H, Ph$\underline{CH}$CHS), 2.90 and 2.95 (2s, 3H, CH_3NCO), 3.24 and 3.34 (2t, J = 7.5 Hz, 2H, CH_2NCO), 3.92 (d, J = 8.1 Hz, 1H, CHCHS), 6.63 and 6.65 (2d, J = 8.4 Hz, 4H, ArH m-to CH and ArH m-to CHSR), 6.75 (d, J = 8.4 Hz, ArH o-to CH), 6.88 (d, J = 8.5 Hz, ArH o-to CHSR); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 12.2 [CH_3 of Et], 13.8, 19.9 (20.0), 25.0 (25.4), 26.4 [CH_2 of Et], 27.8 to 29.3 (7 ×), 29.7 (30.6), 30.7, 32.8 (33.6), 33.7 (35.5) [CH_3N], 47.8 (50.0) [CH_2N], 53.1 [CHEt], 55.9 [CHS], 114.6 (4 ×), 129.8 (2 ×), 130.0 (2 ×), 132.3, 134.0, 154.0, 155.2, 173.8 [CON]; MS *m/e* 392 (M^+ – HOPhCHEt, 22), 287 (19), 240 (62), 211 (86), 142 (62), 114 (100); FABMS calculated for $C_{32}H_{48}NO_3S$ (M^+ – H) 526.3355 found 526.3392.

Synthesis of nonsteroidal thioethers with aryl side chain: 24–27, 35–37 (Fig. 4)

Synthesis of aryl thioethers

The general procedure was described previously (see synthesis of compounds **19–21**), Table 1 shows the conditions use for thioether formation and the yields obtained. The crude thioethers were purified by column chromatography with hexane-EtOAc as eluent.

1-phenylthio-1,2-di(4′-hydroxy phenyl) ethane (24)

Colorless oil; IR *v* (neat) 3350 (OH, phenol); ^{1}H NMR (acetone-d_6, 300 MHz), 3.08 (m, 2H, $\underline{CH_2}CHS$), 4.50 (dd, J_1 = 6.0 Hz and J_2 = 9.1 Hz, 1H, $\underline{CH_2}\underline{CH}S$), 6.64 (d, J = 8.6 Hz, 2H, ArH m-to CH_2), 6.69 (d, J = 8.6 Hz, 2H, ArH m-to CHSR), 6.88 (d, J = 8.4 Hz, 2H, ArH o-to CH_2), 7.12 (d, J = 8.6 Hz, 2H, ArH o-to CHSR), 7.2 to 7.4 (m, 5H, ArH of PhS), 8.08 and 8.22 (2s, 2 × OH); ^{13}C NMR δ (acetone-d_6, 75 MHz), 42.7 [CH_2], 54.7 [CHS], 115.6 (2 ×), 115.7, 115.8, 127.4, 129.6 (2 ×), 130.1 (2 ×), 130.6, 130.9 (2 ×), 132.2 (2 ×), 132.8, 136.8, 156.7, 157.3; MS *m/e* 322 (M^+, 7.6), 215 (100), 213 (100); FABMS calculated for $C_{20}H_{19}O_2S$ (M^+ + H) 323.1106 found 323.1066.

1-(4′-bromo phenylthio)-1,2-di(4′-hydroxy phenyl) ethane (25)

Colorless oil; IR *v* (neat) 3350 (OH, phenol); ^{1}H NMR δ (acetone-d_6, 300 MHz), 3.09 (m, 2H, $\underline{CH_2}CHS$), 4.52 (dd, J_1 = 6.3 Hz and J_2 = 8.8 Hz, 1H, $\underline{CH_2}\underline{CH}S$), 6.65 (d, J = 8.6 Hz, 2H, ArH m-to CH_2), 6.70 (d, J = 8.6 Hz, 2H, ArH m-to CHSR), 6.89 (d, J = 8.6 Hz, 2H, ArH o-to CH_2), 7.12 (d, J = 8.6 Hz, 2H, ArH o-to CHSR), 7.26 (d, J = 8.6 Hz, 2H, Ar o-to S), 7.41 (d, J = 8.7 Hz, 2H, ArH m-to S), 8.24 and 8.41 (2s, 2 × OH); ^{13}C NMR δ (acetone-d_6, 75 MHz), 42.6

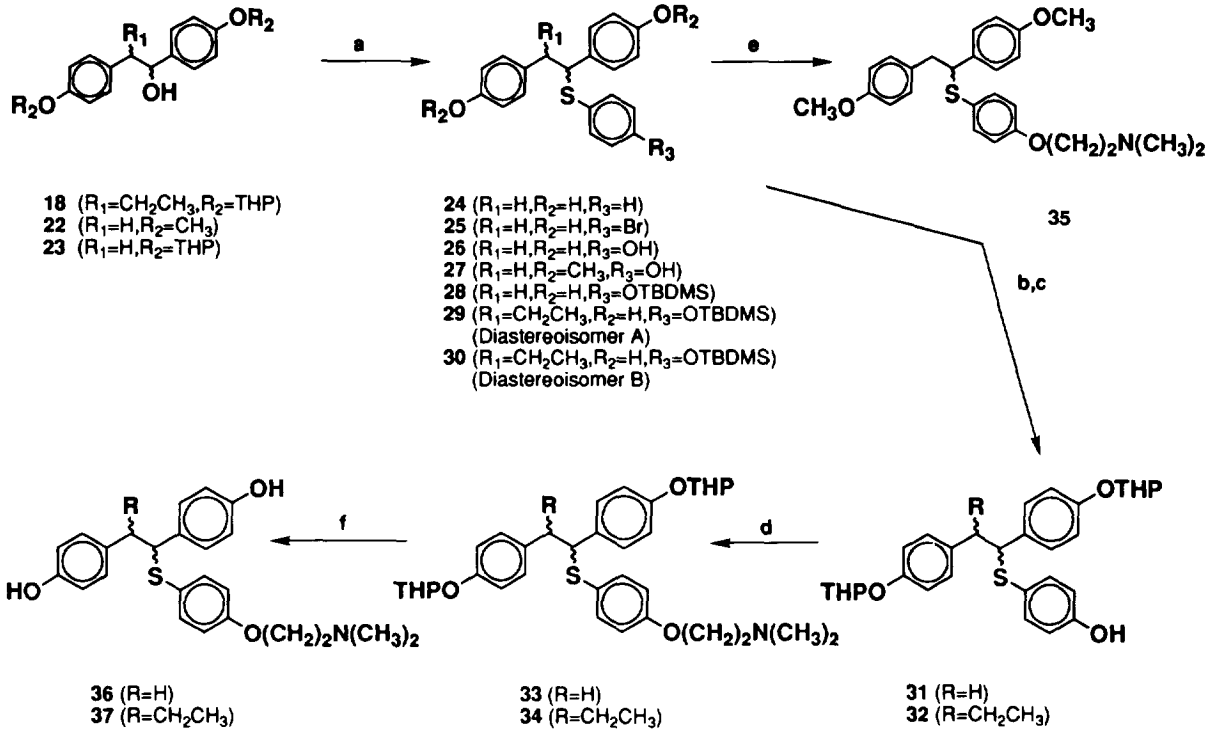


Fig. 4. Synthesis of nonsteroidal thioethers with an aryl side chain (24–27, 35–37). The reagents are (a) HSPhR, ZnI_2, $ClCH_2CH_2Cl$; (b) DHP, *p*-TSA, benzene; (c) $(Bu)_4NF$, THF; (d) 1. NaH, DMF, 40°C; 2. $(CH_3)_2NCH_2CH_2Cl$, 40°C; (e) K_2CO_3, 18-crown-6, $(CH_3)_2NCH_2CH_2Cl$; (f) *p*-TSA, MeOH.

[CH_2], 54.8 [CHS], 115.7 (2 ×), 115.8, 115.9, 120.8, 130.1 (2 ×), 130.4, 131.0 (2 ×), 132.5 (2 ×), 134.0 (3 ×), 136.4, 156.8, 157.4; MS *m/e* 402 (M^+, 2.2), 400 (M^+, 2.0), 295 (39), 293 (39), 213 (100); EIMS calculated for $C_{20}H_{17}O_2SBr$ (M^+) 400.0133 found 400.0142.

1-(4'-hydroxy phenylthio)-1,2-di(4'-hydroxy phenyl) ethane (26)

Colorless oil; IR *v* (neat) 3350 (OH, phenol); ^1H NMR δ (acetone-d_6, 300 MHz), 3.05 (m, 2H, $\underline{CH_2}CHS$), 4.23 (dd, $J_1 = 5.8$ Hz and $J_2 = 9.4$ Hz, 1H, $CH_2\underline{CH}S$), 6.62 (d, J = 8.6 Hz, 2H, ArH m-to CH_2), 6.67 (d, J = 8.6 Hz, 2H, ArH m-to CHSR), 6.75 (d, J = 8.7 Hz, 2H, ArH m-to S), 6.84 (d, J = 8.5 Hz, 2H, ArH o-to CH_2), 7.02 (d, J = 8.5 Hz, 2H, ArH o-to CHSR), 7.20 (d, J = 8.7 Hz, 2H, ArH o-to S), 8.05, 8.19 and 8.50 (3s, 3 × OH); ^{13}C NMR δ (acetone-d_6, 75 MHz), 42.2 [CH_2], 56.5 [CHS], 115.7 (4 ×), 116.6 (2 ×), 125.0, 130.2 (2 ×), 130.9 (3 ×), 133.1, 136.5 (2 ×), 156.5, 157.1, 158.3; MS *m/e* 338 (M^+, 2.8), 230 (20), 212 (100).

1-(4'-hydroxy phenylthio)-1,2-di(4'-methoxy phenyl) ethane (27)

Colorless oil; IR *v* (neat) 3400 (OH, phenol); ^1H NMR δ (acetone-d_6, 300 MHz), 3.1 (m, 2H, $\underline{CH_2}CHS$), 3.70 and 3.73 (2s, 6H, 2 × OCH_3), 4.30 (dd, $J_1 = 5.9$ Hz and $J_2 = 9.4$ Hz, 1H, $CH_2\underline{CH}S$), 6.71 (d, J = 8.7 Hz, 2H, ArH m-to CH_2), 6.75 (d, J = 8.7 Hz, 2H, ArH m-to CHSR), 6.76 (d, J = 8.7 Hz, 2H, ArH m-to S), 6.95 (d, J = 8.6 Hz, 2H, ArH o-to CH_2), 7.12 (d, J = 8.7 Hz, 2H, ArH o-to CHSR), 7.20 (d, J = 8.6 Hz, 2H, ArH o-to S), 8.6 (s, OH); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 41.4 [CH_2], 55.1 [2 × OCH_3], 55.9 [CHS], 113.5 (4 ×), 115.7 (2 ×), 124.9, 129.1 (2 ×), 130.0 (2 ×), 131.2, 133.4, 135.9 (2 ×), 155.8, 157.8, 158.3; MS *m/e* 366 (M^+, 2.7), 245 (53), 241 (100), 225 (57); FABMS calculated for $C_{22}H_{22}O_3S$ (M^+) 366.1290 found 366.1259.

1-(4'-terbutyldimethylsilyloxy phenylthio)-1,2-di(4'-hydroxy phenyl) ethane (28)

Colorless oil; IR *v* (neat) 3380 (OH, phenol); ^1H NMR δ ($CDCl_3$, 300 MHz), 0.17 (s, 6H, $(CH_3)_2$Si of TBDMS), 0.97 (s, 9H, $(CH_3)_3$C of TBDMS), 3.1 (m, 2H, $\underline{CH_2}CHS$), 4.12 (dd, $J_1 = 5.8$ Hz and $J_2 = 9.3$ Hz, 1H, $CH_2\underline{CH}S$), 5.25 and 5.34 (2s, 2H, 2 × OH), 6.62 (d, J = 8.4 Hz, 2H, ArH m-to CH_2), 6.64 (d, J = 8.4 Hz, 2H, ArH m-to CHSR), 6.70 (d, J = 8.4 Hz, 2H, ArH m-to S), 6.82 (d, J = 8.4 Hz, 2H, ArH o-to CH_2), 6.96 (d, J = 8.4 Hz, 2H, ArH o-to CHSR), 7.15 (d, J = 8.4 Hz, 2H, ArH o-to S); ^{13}C NMR δ ($CDCl_3$, 75 MHz), −4.7 [$(CH_3)_2$Si], 18.2 [$(CH_3)_3\underline{C}$]], 25.6 [$(\underline{CH_3})_3C$], 41.5 [CH_2], 55.9 [CHS], 115.0 (4 ×), 120.5 (2 ×), 126.0, 129.4 (2 ×), 130.3 (2 ×), 131.2, 133.4, 135.6 (2 ×), 153.9 154.4, 155.6; MS *m/e* 452 (M^+2.6), 345 (12), 240 (47), 212 (78), 183 (100), 167 (60); EIMS calculated for $C_{26}H_{32}O_3SSi$ (M^+) 452.1842 found 452.1884.

1-(4'-terbutyldimethylsilyloxy phenylthio)-1,2-di(4'-hydroxy phenyl) butane (29 or 30)

Racemic mixture of enantiomers less polar on silica gel: ***29***. Colorless oil; IR *v* (neat) 3370 (OH, phenol); ^1H NMR δ ($CDCl_3$, 300 MHz), 0.13 (s, 6H, $(CH_3)_2$ Si of TBDMS), 0.62 (t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$), 0.94 (s, 9H, $(CH_3)_3$ C of TBDMS), 1.40 and 1.66 (2m, 2H, $\underline{CH_2}CH_3$ two diastereomeric protons), 2.85 (td, $J_1 = 3.5$ Hz and $J_2 = 9.5$ Hz, 1H, $\underline{CH}CHS$), 4.11 (d, J = 9.1 Hz, 1H, $CH\underline{CH}S$), 5.0 (broad, OH), 6.58 (d, J = 8.5 Hz, 2H, ArH m-to CHEt), 6.65 (d, J = 8.3 Hz, 2H, ArH m-to CHSR), 6.78 (d, J = 8.5 Hz, 2H, ArH m-to S), 6.94 (d, J = 8.3 Hz, 2H, ArH o-to CHEt), 6.95 (d, J = 8.4 Hz, 2H, ArH o-to CHSR), 7.02 (d, J = 8.5 Hz, 2H, ArH o-to S); ^{13}C NMR δ (acetone-d_6, 75 MHz), −4.3 [$(CH_3)_2$Si], 12.4 [CH_3 of Et], 15.6 [$(CH_3)_3\underline{C}$], 26.0 [$(\underline{CH_3})_3C$], 27.5 [CH_2 of Et], 53.3 [CH], 61.6 [CHS], 115.5 (2 ×), 115.7 (2 ×), 121.0 (2 ×), 128.3, 130.3 (2 ×), 130.6 (2 ×), 133.4, 134.2, 135.8 (2 ×), 155.9, 156.9, 157.0; MS *m/e* 480 (M^+, 1.7), 345 (51), 240 (100), 211 (60), 183 (100), 167 (89), 107 (100); EIMS calculated for $C_{28}H_{36}O_3SSi$ (M^+) 480.2154 found 480.2085.

Racemic mixture of enantiometers more polar on silica gel: ***30***. Colorless oil; IR *v* (neat) 3370 (OH, phenol); ^1H NMR δ ($CDCl_3$, 300 MHz), 0.15 (s, 6H, $(CH_3)_2$Si of TBDMS), 0.77 (t, J = 7.2 Hz, 3H, $CH_2\underline{CH_3}$), 0.94 (s, 9H, $(CH_3)_3$C of TBDMS), 1.65 and 2.26 (2m, 2H, $\underline{CH_2}CH_3$ two diastereomeric protons), 2.94 (td, $J_1 = 3.8$ Hz and $J_2 = 8.5$ Hz, 1H, $\underline{CH}CHS$), 4.15 (d, J = 8.6 Hz, 1H, $CH\underline{CH}S$), 5.1 (broad, OH), 6.46 (d, J = 8.4 Hz, 2H, ArH m-to CHEt), 6.58 (d, J = 8.4 Hz, 2H, ArH m-to CHSR), 6.62 (d, J = 8.5 Hz, 2H, ArH m-to S), 6.72 (d, J = 8.3 Hz, 2H, ArH o-to CHEt), 6.79 (d, J = 8.3 Hz, 2H, ArH o-to CHSR), 7.04 (d, J = 8.5 Hz, 2H, ArH o-to S); ^{13}C NMR δ (acetone-d_6 75 MHz), −4.3 [$(CH_3)_3$Si], 12.5 [CH_3 of Et], 18.8 [$(CH_3)_3\underline{C}$], 26.1 [$(\underline{CH_3})_3C$], 28.0 [CH_2 of Et], 53.0 [CH], 61.1 [CHS], 115.1 (2 ×), 115.4 (2 ×), 121.2 (2 ×), 127.9, 130.6 (2 ×), 130.8 (2 ×), 133.3, 133.7, 135.9 (2 ×), 156.1, 156.4, 156.5; MS *m/e* 480 (M^+, 1.8), 345 (46), 240 (68), 211 (30), 183 (100), 167 (49); EIMS calculated for $C_{28}H_{36}O_3SSi$ (M^+) 480.2154 found 480.2189.

Synthesis of diTHP-hydroxy thioethers ***31***, ***32*** *(general procedure)*

The diphenolic compound **28** or **29** (0.96 mmol) was dissolved in dry CH_2Cl_2 (20 ml), 3,4-dihydro-2H-pyran (0.87 ml, 9.6 mmol), and a catalytic amount of p-TSA (17 mg) was added. The mixture was stirred at 0°C under argon for 1 h, poured into a saturated $NaHCO_3$ solution, extracted with EtOAc, washed with water, and dried over $MgSO_4$. The crude product was roughly purified by chromatography before the next

step (hexane-EtOAc, 90:10 as eluent). The diTHP derivative (0.63 mmol) was then dissolved in dry THF (30 ml), and tetrabutylammonium fluoride (0.19 ml of 1.0 M solution in tetrahydrofuran) was added dropwise at room temperature and allowed to react for 3 h at this temperature. The mixture was immediately purified by column chromatography with hexane-EtOAc 80:20 as eluent.

1-(4'-hydroxy phenylthio)-1,2-di{4'-[(tetrahydro-2"H-pyran-2"-yl)oxy]phenyl} ethane (31)

Colorless oil (68% yield); IR ν (neat) 3340 (OH, phenol); ^{1}H NMR δ (acetone-d_6, 300 MHz), 1.5–2.0 (m, 12H, 6 × CH_2 of THP), 3.12 (m, 2H, $\underline{CH_2}CHS$), 3.53 and 3.80 (2m, 4H, 2 × CH_2O of THP), 4.32 (t_{app}, J = 7.6 Hz, 1H, $CH_2\underline{CH}S$), 5.32 and 5.36 (2m, 2H, 2 × CH of THP), 6.75 (d, J = 8.5 Hz, 2H, ArH m-to S), 6.84 (d, J = 8.6 Hz, 2H, ArH m-to CH_2), 6.89 (d, J = 8.5 Hz, 2H, ArH m-to CHSR), 6.97 (d, J = 8.3 Hz, 2H, ArH o-to CH_2), 7.13 (d, J = 8.7 Hz, 2H, ArH o-to CHSR), 7.20 (d, J = 8.5 Hz, 2H, ArH o-to S) 8.49 (s, OH); MS *m/e* 338 (M^+ – 2 DHP, 0.8), 231 (3.8), 212 (100).

1-(4'-hydroxy phenylthio)-1,2-di{4'-[(tetrahydro-2"H-pyran-2"-yl)oxy]phenyl} butane (32)

White solid (63% yield); mp 159–161°C; IR ν (KBr) 3420 (OH, phenol); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.60 (t, J = 7.2 Hz, 3H, $CH_2\underline{CH_3}$), 1.5–2.1 (m, 14H, 6 × CH_2 of THP and $\underline{CH_2}CH_3$), 2.85 (td, J_1 = 2.9 Hz and J_2 = 10.3 Hz, 1H, $\underline{CH}CHS$), 3.62 and 3.95 (2m, 4H, 2 × CH_2O of THP), 4.09 and 4.10 (2d, J = 9.2 Hz, 1H, $CH\underline{CH}S$), 5.0 (broad, OH), 5.35 and 5.41 (2m, 2H, 2 × CH of THP), 6.52 (d, J = 8.6 Hz, 2H, ArH m-to S), 6.85–7.10 (m, 10H, ArH); MS *m/e* 366 (M^+ – 2DHP, 0.7), 240 (69), 231 (32); FABMS calculated for $C_{32}H_{39}O_5S$ (M^+ + H) 535.2518 found 535.2508.

Synthesis of 2-dimethylamino ethyl ether derivatives 33, 34 and 35

Alkylation of phenol 27 to 35 (method 1). Dimethoxyphenol 27 (0.55 mmol) was dissolved in DMF (Analar, BDH, HPLC grade, water: 0.1%). K_2CO_3 (1.2 mmol), 18-Crown-6 (0.11 mmol) and 2-dimethylamino ethyl chloride hydrochloride (0.60 mmol) was added. The mixture was stirred for 12 h at room temperature and finally refluxed for 1 h. Water was then added and the reaction mixture extracted with EtOAc. The organic phase was dried over $MgSO_4$, evaporated to dryness, and the crude product was purified by column chromatography with EtOAc-methanol 80:20 as eluent.

1-[4'-(2"-dimethylamino ethoxy) phenylthio]-1,2-di(4'-methoxy phenyl) ethane (35)

Colorless oil (71% yield); IR ν (neat) no phenolic band; ^{1}H NMR δ ($CDCl_3$, 300 MHz), 2.34 (s, 6H, $N(CH_3)_2$), 2.73 (t, J = 5.7 Hz, 2H, CH_2N), 3.10 (m, 2H, $\underline{CH_2}CHS$), 3.73 and 3.76 (2s, 6H, 2 × OCH_3), 4.02 (t, J = 5.7 Hz, 2H, OCH_2), 4.36 (dd, J_1 = 6.1 Hz and J_2 = 9.2 Hz, 1H, $CH_2\underline{CH}SR$), 6.71 (d, J = 8.0 Hz, 2H, ArH m-to CH_2), 6.73 (d, J = 8.0 Hz, 2H, ArH m-to CHSR), 6.76 (d, J = 7.6 Hz, 2H, ArH m-to S), 6.89 (d, J = 8.6 Hz, 2H, ArH o-to CH_2), 7.04 (d, J = 8.7 Hz, 2H, ArH o-to CHSR), 7.19 (d, J = 8.7 Hz, 2H, ArH o-to S); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 41.6 [$\underline{CH_2}CHS$], 45.8 [$(CH_3)_2N$], 55.2 [2 × OCH_3], 55.9 [CHS], 58.1 [CH_2N], 65.9 [OCH_2], 113.5 (4 ×), 114.9 (2 ×), 125.5, 129.1 (2 ×), 130.1 (2 ×), 131.2, 133.5, 135.6 (2 ×), 158.0 158.5, 158.6; MS *m/e* 437 (M^+, 3.6), 316 (9), 241 (100); FABMS calculated for $C_{26}H_{32}NO_3S$ (M^+ + H) 438.2103 found 438.2121.

General procedure for alkylation of phenol 31 or 32 to 33 or 34 (method 2). A mixture of phenol 31 or 32 (0.25–0.43 mmol), dry DMF (4–8 ml), and sodium hydride (3.75–6.50 mmol of NaH 60% dispersion in mineral oil) was stirred at 40°C for 0.5 h in dry condition. The mixture was then cooled to 25°C and 2-dimethylamino ethyl chloride hydrochloride (1.25–2.15 mmol) added. After stirring for 0.5 h at 40°C, the reaction was cooled to 0°C and quenched by adding water and a saturated $NaHCO_3$ solution. The resulting aqueous phase was extracted with EtOAc and dried over $MgSO_4$. After evaporation of the solvent, the crude compound was purified by column chromatography with EtOAc–methanol 80:20 as eluent.

1-[4'-(2"-dimethylamino ethoxy)phenylthiol]-1,2-di{4'-[(tetrahydro-2"H-pyran-2"-yl)oxy]phenyl}ethane (33)

Colorless oil (78% yield); IR ν (neat) no phenolic band; ^{1}H NMR δ (acetone-d_6, 300 MHz), 1.5–2.0 (m, 12H, 6 × CH_2 of THP), 2.27 (s, 6H, $N(CH_3)_2$, 2.67 (t, J = 5.9 Hz, 2H, CH_2N), 3.13 (m, 2H, $\underline{CH_2}CHS$), 3.53 and 3.81 (2m, 4H, 2 × CH_2O of THP), 4.05 (t, J = 5.9 Hz, 2H, OCH_2), 4.38 (dd, J_1 = 6.6 Hz and J_2 = 8.6 Hz, 1H, $CH_2\underline{CH}S$), 5.32 and 5.35 (2t, J = 3 Hz, 2H, 2 × CH of THP), 6.83 (d, J = 8.8 Hz, 2H, ArH m-to S), 6.85 (d, J = 8.8 Hz, 2H, ArH, m-to CH_2), 6.90 (d, J = 8.6 Hz, 2H, ArH m-to CHSR), 6.98 (J = 8.6 Hz, 2H, ArH o-to CH_2), 7.15 (d, J = 8.6 Hz, 2H, ArH o-to CHSR), 7.27 (d, J = 8.7 Hz, 2H, ArH o-to S); ^{13}C NMR δ (acetone-d_6, 75 MHz), 19.7 (2 ×), 26.0 (2 ×), 31.2 (2 ×), 42.2 [$\underline{CH_2}CHS$], 46.2 [$(CH_3)_2N$], 56.0 [CHS], 58.9 [CH_2N], 62.5 [2 × CH_2O of THP], 67.2 [CH_2O], 97.2 (2 × OCHO of THP], 115.8 (4 ×), 116.9 (2 ×), 126.5, 130.0 (2 ×), 130.8 (2 ×), 133.0, 135.2, 136.0 (2 ×), 156.6, 157.2, 159.7; MS *m/e* 577 (M^+, 0.1), 302 (3.0), 296 (7.7), 212 (100); FABMS calculated for $C_{34}H_{44}NO_5S$ (M^+ + H) 578.2940 found 578.2905.

1-[4'-(2"-dimethylamino ethoxy) phenylthio]-1,2-di {4'-[(tetrahydro-2"H-pyran-2"-yl)oxy]phenyl}butane (34).

Colorless oil (87% yield); IR ν (neat) no phenolic band; ^{1}H NMR δ (CD_3OD, 300 MHz), 0.59 (t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$), 1.3–2.1 (m, 14H, 6 × CH_2 of THP and $\underline{CH_2}CH_3$), 2.32 (s, 6H, $N(CH_3)_2$), 2.73 (t, J = 5.4 Hz, 2H, CH_2N), 2.85 (td, J_1 = 2.9 Hz and J_2 = 9.9 Hz, 1H, CHCHS), 3.60 and 3.92 (2m, 4H, 2 × CH_2O of THP), 4.00 (t, J = 5.5 Hz, 2H, OCH_2), 4.20 (d, J = 9.0 Hz, 1H, CHS), 5.34 and 5.40 (2m, 2H, 2 × CH of THP), 6.67 (d, J = 8.7 Hz, 2H, ArH m to S), 6.86 (d, J = 8.7 Hz, 2H, ArH m- to CH_2), 6.97 (d, J = 8.4 Hz, 4H, ArH m-to CHSR and o-to CH_2, 7.03 (d, J = 8.6 Hz, 2H, ArH o-to CHSR), 7.10 (d, J = 8.4 Hz, 2H ArH o-to S); ^{13}C NMR δ (CD_3OD, 75 MHz), 12.4 [CH_3 of Et] 20.2 (2 ×), 26.4 (2 ×), 28.0 [CH_2 of Et], 31.7 (2 ×), 45.8 [$(CH_3)_2N$], 54.0 [CHCHS], 59.0 [CH_2N], 62.3 [2 × CH_2O of THP], 63.3 [CHS], 66.5 [OCH_2], 98.1 2 × OCHO of THP], 115.7 (2 ×), 117.0 (2 ×), 117.2 (2 ×), 127.6, 130.5 (2 ×), 130.7 (2 ×), 136.2, 136.6 (2 ×), 136.9 157.3 (2 ×), 159.7; MS *m/e* 386 (M^+-THPOPhCHEt, 0.3), 324 (0.8), 302 (3.5), 240 (63), 58 (100); FABMS calculated for $C_{36}H_{48}NO_5S$ (M^+ + H) 606.3253 found 606.3223.

Typical procedure for hydrolysis of THP derivatives 33, 34 to phenols 36, 37

To the THP derivatives **33**, **34** (0.18–0.35 mmol) dissolved in MeOH (40–70 ml), p-TSA (0.24–0.52 mmol) was added and the resulting solution was stirred at room temperature for 2 h. The reaction was quenched by the addition of water, and methanol was evaporated under reduced pressure. The mixture was poured into a saturated $NaHCO_3$ solution, extracted with EtOAc, washed with water, and dried over $MgSO_4$. After evaporation of solvent, the crude compound was purified by column chromatography with EtOAc–methanol 60:40 as eluent.

1-[4'-(2"-dimethylamino ethoxy) phenylthio]-1,2-di(4'-hydroxy phenyl) ethane (36)

Colorless oil (75% yield); IR ν (neat) 3307 (OH, phenol); ^{1}H NMR δ (acetone-d_6, 300 MHz), 2.26 (s, 6H, $N(CH_3)_2$), 2.67 (t, J = 5.9 Hz, 2H, CH_2N), 3.05 (m, 2H, CH_2CH), 4.06 (t, J = 5.9 Hz, 2H, OCH_2), 4.29 (dd, J_1 = 5.9 Hz and J_2 = 9.2 Hz, CH_2CHS), 6.63 (d, J = 8.6 Hz, 2H, ArH m-to CH_2), 6.68 (d, J = 8.6 Hz, 2H ArH m-to CHSR), 6.84 (d, J = 8.9 Hz, 2H, ArH o-to CH_2), 6.85 (d, J = 8.5 Hz, 2H, ArH m-to S), 7.04 (d, J = 8.6 Hz, ArH o-to CHSR), 7.27 (d, J = 8.9 Hz, 2H, ArH o-to S); ^{13}C NMR δ (acetone-d_6, 75 MHz), 42.3 [CH_2CHS], 46.1 [$(CH_3)_2N$], 56.3 [CHS], 58.8 [CH_2N], 67.2 [CH_2O], 115.7 (6 ×), 126.7 130.2 (2 ×), 130.9 (2 ×), 133.1, 136.0 (3 ×), 156.6, 157.2, 159.7; MS *m/e* 409 (M^+, 0.1), 302 (0.7), 212 (100); FABMS calculated for $C_{24}H_{28}NO_3S$ (M^+ + H) 410.1790 found 410.1804.

1-[4'-(2"-dimethylamino ethoxy) phenylthio]-1,2-di(4'-hydroxy phenyl) butane (37)

Colorless oil (83% yield); IR ν (neat), 3330 (OH, phenol); ^{1}H NMR δ (acetone-d_6, 300 MHz), 0.60 and 0.74 (2t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$, mixture of two diastereoisomers, 65:35), 1.4 and 1.6 (2m, 2H, $\underline{CH_2}CH_3$), 2.24 (s, 6H, $N(CH_3)_2$), 2.63 (t, J = 5.9 Hz, 2H, CH_2N), 2.87 and 2.92 (2td, J_1 = 3.5 Hz and J_2 = 7.5 Hz, 1H, CHCHS, 65:35), 3.99 (t, J = 6.0 Hz, 2H, OCH_2), 4.27 and 4.31 (2d, J = 9.4 Hz, 1H, CHCHS, 65:35), 6.5–7.2 (m, 12H, ArH); ^{13}C NMR δ (acetone-d_6, 75 MHz), 12.4 [CH_3 of Et], 27.5 and 27.9 [CH_2 of Et, 65:35], 46.1 [$(CH_3)_2N$], 53.0 and 53.5 [CHCHS, 35:65], 58.8 [CH_2N], 61.1 and 61.7 [CHS, 35:65], 67.1 [OCH_2], 115.0 (2 ×), 115.5 (2 ×), 115.7 (2 ×), 127.2 130.3 (2 ×), 130.6 (2 ×), 133.4, 134.1, 135.8 (2 ×), 156.4 (2 ×), 157.1 (2 ×), 159.3 (2 ×); MS *m/e* 437 (M^+, 0.5), 302 (11), 240 (61); FABMS calculated for $C_{26}H_{32}NO_3S$ (M^+ + H) 438.2103 found 438.2076.

Synthesis of thioether estradiol derivatives: 43–45, and 47 (Fig. 5)

Synthesis of 3-acetoxy-1,3,5(10) estratriene-17-one (39)

Estrone (**38**) (55.6 mmol) was dissolved in a solution of pyridine and acetic anhydride (800 ml of solution 50:50) at 0°C. The mixture was stirred at room temperature for 18 h. At this time, the reaction was poured into a mixture of water and ice. The resulting white solid was filtered, washed with water, and dried under a vacuum pump for 1 or 2 days. White solid (98% yield), mp 125–127°C (lit. [34] 122–124°C); IR ν (KBr) 1750 (C=O, ester), 1720 (C=O, ketone); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.91 (s, 3H, 18-CH_3), 2.28 (s, 3H, CH_3COO), 2.90 (m, 2H, 6-CH_2), 6.81 (s_{app}, 1H, 4-CH), 6.85 (dd, J_1 = 2.2 Hz and J_2 = 8.6 Hz, 1H, 2-CH), 7.29 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* 312 (M^+58), 270 (100).

Synthesis of 3-acetoxy-1,3,5(10)-estratriene-6,17-dione (40)

According to the procedure described by Takagi *et al.* [35] a suspension of chromium (VI) oxide (16 mmol) in CH_2Cl_2 (25 ml) was stirred at 0°C. Then 3,5-dimethyl pyrazole was added, the suspension was stirred for 15 min, and 3-acetoxy estrone (**39**) dissolved in CH_2Cl_2 (2.5 ml) was added to the mixture and stirred for 17 h at room temperature. At this time, the reaction mixture was filtered through silica gel using hexane-acetone 70:30 as eluent. A second column chromatography with hexane–acetone 75:25 was performed to obtain a white solid (30% yield), mp 192–195°C; IR ν (KBr) 1740 (C=O, ester), 1710

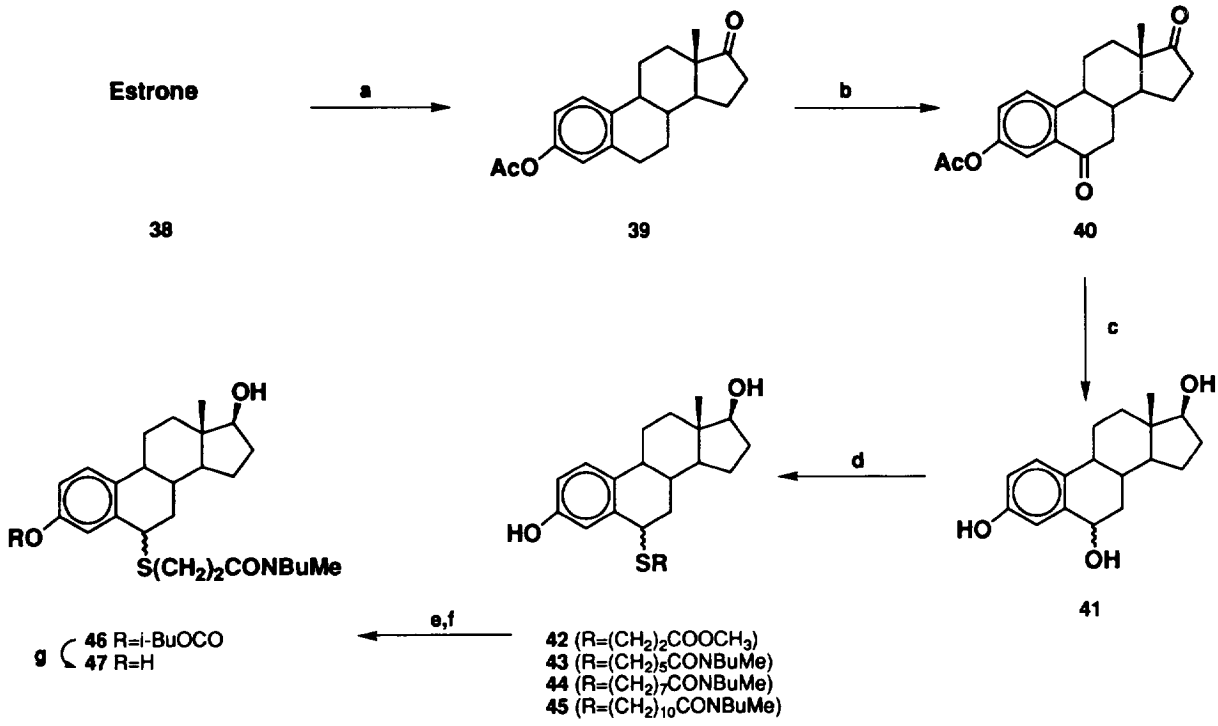


Fig. 5. Synthesis of thioether estradiol derivatives with an alkylamide side chain (43–45 and 47). The reagents are (a) $CH_3COOCOCH_3$, pyr.; (b) CrO_3, CH_2Cl_2, 3,5-dimethyl pyrazole; (c) $LiAlH_4$, THF; (d) HSR, ZnI_2, $ClCH_2CH_2Cl$; (e) KOH, H_2O, MeOH; (f) 1. ClCOOi-Bu, $N(Bu)_3$; 2. HNBuMe; (g) K_2CO_3, H_2O, MeOH.

(C=O, ketone), 1650 (C=O, conjugated ketone); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.91 (s, 3H, 18-CH_3), 2.30 (s, 3H, CH_3COO), 7.27 (dd, $J_1 = 2.4$ Hz and $J_2 = 8.5$ Hz, 1H, 2-CH), 7.44 (d, J = 8.6 Hz, 1H, 1-CH), 7.75 (d, J = 2.4 Hz, 1H, 4-CH); MS *m/e* 326 (M^+, 26) 284 (100); EIMS calculated for $C_{20}H_{22}O_4$ (M^+) 326.1518 found 326.1499.

Synthesis of 3,6(α,β), 17β-trihydroxy-1,3,5(10)-estratriene (41)

Under anhydrous conditions, compound 40 (0.15 mmol) was dissolved in dry THF (5 ml), and $LiAlH_4$ (0.45 mmol) was added to the solution. After a few minutes, the reaction was quenched by adding water, and THF was evaporated under reduced pressure. The resulting white solid was filtered, washed with water, and dried under vacuum pump for 1 or 2 days to produce a white solid (52% yield), mp 210–212°C; IR *v* (KBr) 3300 (OH, phenol and alcohol); ^{1}H NMR δ (CD_3OD, 300 MHz), 0.77 (s, 3H, 18-CH_3), 3.66 (t, J = 8.4 Hz, 1H, 17-CH), 4.70 (dd, $J_1 = 6.4$ Hz and $J_2 = 10.4$ Hz, 1H, 6-CH), 6.61 (dd, J = 2.7 Hz and $J_2 = 8.4$ Hz, 1H, 2-CH), 6.97 (d, J = 2.6 Hz, 1H, 4-CH), 7.08 (d, J = 8.6 Hz, 1H, 1-CH); MS *m/e* 288 (M^+, 16), 270 (100); EIMS calculated for $C_{18}H_{24}O_3$ (M^+) 288.1725 found 288.1746.

Synthesis of estradiol 6-thioether derivatives (42–45)

The general procedure for thioether formation (ZnI_2, HSR), previously described, was used for the synthesis of estradiol 6-thioether derivatives 42–45. Table 1 shows the conditions used for these transformations and the yield achieved.

Methyl 4-(3',17'β-dihydroxy-1',3',5'(10')-estratriene-6'(α,β)-yl)-4-thiabutanoate (42)

White foam; IR *v* (film) 3280 (OH, phenol and alcohol), 1680 (C=O, chelated ester); ^{1}H NMR δ (CD_3OD, 300 MHz), 0.74 and 0.80 (2s, 3H, 18'-CH_3, two isomers 50:50), 3.61 and 3.65 (2s, 3H, OCH_3), 3.7 (m, 1H, 17'-CH), 4.10 (t_{app}, J = 8.5 Hz, 0.5H, 6'β-CH), 4.17 (d, J = 3.3 Hz, 0.5H, 6'α-CH) 6.68 (dd, $J_1 = 2.1$ Hz and $J_2 = 8.4$ Hz, 1H 2'-CH), 6.81 and 7.12 (2d, J = 2.5 Hz, 1H, 4'-CH, two isomers) 7.08 (d, J = 8.5 Hz, 1H, 1'-CH); MS *m/e* 390 (M^+, 5.0), 303 (16), 270 (89); EIMS calculated for $C_{22}H_{30}O_4S$ (M^+) 390.1865 found 390.1916.

N-butyl, N-methyl 7-(3',17'β-dihydroxy-1',3',5'(10')-estratriene-6'(α,β)-yl)-7-thiaheptamide (43)

White foam; IR *v* (film) 3200 (OH, phenol and alcohol), 1600 (C=O, amide); ^{1}H NMR δ (acetone-d_6, 300 MHz), 0.75 and 0.81 (2s, 3H, 18'-CH_3, two isomers 50:50), 0.91 and 0.94 (2t, J = 7.3 Hz, 3H, $N(CH_2)_3\underline{CH_3}$), 2.88 and 3.02 (2s, 3H, NCH_3), 3.35 (m, 2H, $\underline{NCH_2}$) 3.68 (m, 1H, 17'-CH), 4.05 (t_{app}, J = 8.4 Hz, 0.5H, 6'β-CH), 4.13 (d, J = 4.0 Hz, 0.5H, 6'α-CH), 6.65 (dd, $J_1 = 2.5$ Hz and $J_2 = 8.4$ Hz, 1H, 2'-CH), 6.85 and 7.20 (2d, J = 2.4 Hz, 1H, 4'-CH, two isomers), 7.10 (d, J = 8.4 Hz, 1H, 1'-CH), 8.4 and 8.5

(2s, 2H, OH); MS *m/e* 487 (M^+, 0.3), 270 (100), 216 (78); EIMS calculated for $C_{29}H_{45}NO_3S$ (M^+) 487.3120 found 487.3106.

N-butyl, N-methyl 9-(3',17'β-dihydroxy-1',3',5'(10')-estratriene-6'(α,β)-yl)-9-thianonamide (44)

White foam, IR *v* (film) 3260 (OH, phenol and alcohol), 1607 (C=O, amide); ^{1}H NMR δ (acetone-d_6, 300 MHz), 0.75 and 0.82 (2s, 3H, 18'-CH_3, two isomers 50:50), 0.91 and 0.94 (2t, J = 7.3 Hz, $N(CH_2)_3\underline{CH_3}$), 2.87 and 3.02 (2s, 3H, NCH_2), 3.35 (m, 2H, NCH_2), 3.67 (m, 1H, 17'-CH), 4.08 (t_{app}, J = 8.5 Hz, 0.5H, 6'β-CH), 4.14 (d, J = 3.6 Hz, 0.5H, 6'α-CH), 6.64 (dd, J_1 = 2.4 Hz and J_2 = 8.5 Hz, 1H, 2'-CH), 6.87 and 7.18 (2d, J = 2.6 Hz, 1H, 4'-CH, two isomers), 7.09 and 7.10 (2d, J = 8.5 Hz, 1H, 1'-CH); MS *m/e* 515 (M^+, 0.2), 270 (100), 211 (17), 157 (52); EIMS calculated for $C_{31}H_{49}NO_3S$ (M^+) 515.3433, found 515.3384.

N-butyl, N-methyl 12-(3',17'β-dihydroxy-1',3',5'(10')-estratriene-6'(α,β)-yl)-12-thiadodecamide (45)

White foam; IR *v* (neat) 3300 (OH, phenol and alcohol), 1620 (C=O, amide); ^{1}H NMR ($CDCl_3$, 300 MHz), 0.74 and 0.82 (2s, 3H, 18'-CH_3, two isomers 50:50), 0.90 and 0.93 (2t, J = 7.3 Hz, 3H, $N(CH_2)_3\underline{CH_3}$), 2.91 and 2.96 (2s, 3H, NCH_3), 3.25 and 3.35 (2t, J = 7.5 Hz, 2H, NCH_2), 3.70 (m, 1H, 17'-CH), 4.01 (t_{app}, J = 8.9 Hz, 0.5H, 6'β-CH) 4.05 (d, J = 4.9 Hz, 0.5H, 6'α-CH), 6.69 (m, 1H, 2'-CH), 6.85 and 7.18 (2d, J = 2.4 Hz, 1H, 4'-CH, two isomers), 7.07 and 7.09 (2d, J = 8.5 Hz, 1H, 1'-CH); ^{1}H-NMR ($CDCl_3$, 300 MHz) (only one isomer, 6'α-side chain), 0.75 (s, 3H, 18'-CH_3), 0.92 and 0.95 (2t, J = 7.3 Hz, 3H, $N(CH_2)_3\underline{CH_3}$), 2.94 and 2.98 (2s, 3H, NCH_3), 3.27 and 3.38 (2t, J = 7.4 Hz, 2H, NCH_2), 3.73 (t, J = 8.5 Hz, 1H, 17'-CH), 4.04 (t_{app}, J = 8.5 Hz, 1H, 6'β-CH), 6.72 (dd, J_1 = 2.5 Hz and J_2 = 8.3 Hz, 1H, 2'-CH), 7.10 (d, J = 8.5 Hz, 1H, 1'-CH), 7.19 (d, J = 2.5 Hz, 1H, 4'-CH); MS *m/e* 557 (M^+, 3.0), 286 (21), 270 (100), 254 (13), 157 (28), 114 (33); FABMS calculated for $C_{34}H_{54}NO_3S$ ($M^+ - H$) 556.3824 found 556.3851.

Synthesis of N-butyl, N-methyl 4-(3'-17'β-dihydroxy 1',3',5'(10')-estratriene-6'(α,β)-yl)-4-thiabutanamide (47) (procedure for hydrolysis of ester, amide formation and hydrolysis of the carbonate group, 42 to 46 to 47)

To a solution of ester 42 (910 mg, 2.33 mmol) in MeOH (250 ml) an aqueous solution of KOH 10%, w/v (125 ml) was added, and the mixture was refluxed under an argon atmosphere for 5 h. Thereafter water was added and MeOH was evaporated under vacuum. The resulting solution was acidified with HCl and extracted with EtOAc. The organic phase was washed with water and brine and dried over $MgSO_4$. Without purification, the crude carboxylic acid was dissolved in dry CH_2Cl_2 (270 ml) and tributylamine (2.2 ml, 9.3 mmol). After cooling the mixture at −10°C, isobutyl chloroformate (1.2 ml, 9.3 mmol) was added and allowed to react for 30 min. At this time, *N*-methylbutylamine in excess (5.5 ml, 47 mmol) was added, and the cooling bath was removed. After 4 h, CH_2Cl_2 was added and the organic phase was washed with HCl (1N) and dried over $MgSO_4$. The solvent was removed and the crude amide purified by column chromatography (hexane–EtOAc, 7:3) to give, in 71% yield, the amide **46** with a carbonate group in 3-position. This carbonate derivative **46** (600 mg, 1.1 mmol) was dissolved in MeOH (170 ml). Then K_2CO_3 (1%, w/v) in aqueous MeOH (25:75, v/v) (170 ml) was added, and the resulting solution was stirred at room temperature for 6 h. The reaction mixture was acidified with HCl (1N), and MeOH was evaporated under vacuum. The residue was extracted with EtOAc and the organic phase dried ($MgSO_4$) and evaporated. Purification was done by column chromatography (hexane–acetone, 7:3) to lead the free phenolic derivative 47 in 60% yield. White foam IR *v* (neat) 3300 (OH, phenol and alcohol), 1625 (C=O, amide); ^{1}H NMR δ ($CDCl_3$, 300 MHz), 0.74 and 0.82 (2s, 3H, 18'-CH_3, two isomers 50:50), 0.91 and 0.94 (2t, J ~ 7 Hz, $N(CH_2)_3\underline{CH_3}$), 2.88, 2.89, 2.94 and 2.97 (4s, 3H, NCH_3), 3.15, 3.25 and 3.35 (2t and q_{app}, 2H, NCH_2), 3.74 (m, 1H, 17'-CH), 4.05 and 4.10 (t_{app} and d, 1H, 6'-CH), 6.70 (dd, J_1 = 2.8 Hz and J_2 = 8.5 Hz, 1H, 2'-CH), 6.84 and 7.16 (2d, J = 2.5 Hz, 1H, 4'-CH), 7.09 and 7.12 (2d, J = 8.3 Hz, 1H, 1'-CH); MS *m/e* 445 (M^+, 0.6), 303 (2.6), 270 (100), 174 (56), 114 (53); FABMS calculated for $C_{26}H_{40}NO_3S$ ($M^+ + H$) 446.2731 found 446.2761.

Biological Assays

Preparation of compounds for biological assays

Before submitting new synthesized compounds for biological evaluation, a final silica gel chromatography was performed with HPLC-grade solvents, and central fractions were carefully evaporated to dryness. Purity was checked by HPLC (Waters Associates, Milford, MA) with a reverse-phase column (C-18 Nova-pak, 4 μM, 0.5 cm × 10 cm), and determined to be greater than 98%.

Estrogen receptor binding assay

Apparent affinities to the synthesized compounds for the estrogen receptor were determined by competition binding with [^{3}H] estradiol (E_2) to the rat uterine cytosol receptor according to Asselin and Labrie [36]. Incubations were performed at 25°C for 3 h and non-specific binding determined using an excess (1000 nM) of radioinert estradiol. The apparent affinities were expressed as relative binding affinity (RBA) or in % of displacement at a concentration of 10 μM. Calculations were performed according to the following equations: (1) RBA = 100 × IC_{50} of E_2/IC_{50} of tested compound,

where IC_{50} is the concentration of E_2 or tested compound that inhibits [^{3}H]E_2 binding by 50%, the RBA of E_2 being taken as 100%; and (2) % of displacement at $10\,\mu M = 100 \times [(A - B)/(A - C)]$, where: A is [^{3}H]$E_2$ bound (without tested compound), B is [^{3}H]E_2 bound (with $10\,\mu M$ of tested compound), C is [^{3}H]E_2 bound (with $1\,\mu M$ unlabeled E_2); the % of E_2 being taken as 100%.

Proliferative and antiproliferative ZR-75-1 cell assay

The ZR-75-1 human breast cancer cells were harvested in their exponential growth with 0.05% trypsin–0.02% EDTA (w/v) and resuspended in RPMI 1640 medium without phenol red supplement with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 ng insulin per ml, 15 mM HEPES 100 IU penicillin per ml, 100 μg streptomycin sulfate per ml, and 5% (v/v) dextran-coated charcoal-treated fetal bovine serum (SD medium). The cells were plated in Falcon 24-well tissue culture plates (2 cm^2/well) with 10,000 cells per dish and allowed to adhere to the substrate for 3 days. We then added estradiol (0.1 nM) or indicated concentrations of the tested compound from concentrated stock solutions in 99% redistilled ethanol into fresh SD medium. The cells were then incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 9 days with a change of medium every 2 days. After the incubation period, cell growth was assessed by measuring DNA content using a modification of the Fiszer–Szafarz method [37] as previously described [38].

Calculations were performed according to the following equations and expressed as percentages: (a) stimulation of cell proliferation or estrogenic activity = $[(B - A)/A] \times 100$; (b) inhibition of basal cell proliferation or antiproliferative activity = $[(A - B)/A] \times 100$; and (c) inhibition of estradiol(E_2)-stimulated cell proliferation or antiestrogenic activity = $100 - [(D - B)/(C - A)] \times 100]$, where A is the DNA content of cells incubated with control medium (μg), B is the DNA content of cells treated with the tested compound (μg), C is the DNA content of E_2-stimulated cells (μg) and D is the DNA content of E_2-stimulated cells treated with the tested compound (μg).

RESULTS

Chemistry

*Synthesis of nonsteroidal thioethers with an alkylamide side chain: **19–21** (Fig. 3)*

The synthesis of nonsteroidal thioethers with an alkylamide side chain **19–21** was performed using desoxyanisoin (**15**) as starting material (Fig. 3). The racemic dimethoxy ethyl ketone **16** (R:S/50:50) was obtained by alkylation of the α-ketone position with ethyl bromide and LDA as base. The di-THP ethyl ketone **17** was obtained in two steps: (i) di-demethylation of **16** with pyr.HCl according to the procedure of Buu-Hoi *et al.* [33]; and (ii) successive tetrahydropyranylation (dihydropyran, *p*-TSA) of phenolic groups. The di-THP ethyl alcohol **18** was obtained by sodium borohydride ($NaBH_4$) reduction of di-THP ethyl ketone **17**. The global yield for the synthesis of starting alcohol **18** was good (31%, four steps).

The thioether derivatives **19–21** were obtained by the reaction of an activated alcohol **18** and a mercapto group (HSR) in the presence of zinc iodide. Herein the two starting mercapto side chains ($HS(CH_2)_nCONBuMe$, $n = 7$, 10) were obtained from corresponding bromoacid as described [32]. This simple, efficient catalyzed coupling reaction was first reported by Guindon *et al.* [39] and recently extended to thioether with alkylamide side chain by our group [32]. During this reaction, we observed the partial hydrolysis of the THP-protective group. In fact, this group was cleaved by zinc iodide to give the suitable phenolic group. The THP-ether not cleaved was easily hydrolyzed to a phenolic compound by p-TSA in MeOH [40] (see Table 1 for yields of compounds **19–21**). This thioether formation provided a mixture of diastereoisomers A and B (Fig. 3), each of them being a racemic mixture of enantiomers for a total of four isomers. Separation of diastereoisomers A and B was possible by conventional silica gel flash chromatography. As expected no optical activity $[\alpha]_D$ was measured for A or B supporting a racemic mixture of enantiomers. Thus, for clarity of discussion in the following text, the diastereoisomer A corresponds to enantiomeric mixture of S,S and R,R, and diastereoisomer B corresponds to enantiomeric mixture of S,R and R,S. For compounds with a longer side chain ($n = 10$), the proportion of diastereoisomers A and B was 59:41, for **20** and **21**, respectively. For a compound with a smaller side chain ($n = 7$), diasteroisomers A and B were also observed, but only one (A, compound **19**) was recovered and used for biological analysis.

The new compounds **19–21** resulting from the coupling reaction were characterized by spectroscopic means. The COSY and HETCOR analyses were particularly useful for attribution of NMR signals. In IR, the amide band was always present in these thioether compounds. In ^{1}H and ^{13}C NMR, the signals of the CHS system were characteristic and appeared at 3.78, 3.87, 3.94 ppm and 53.16, 53.26, 53.10 ppm, respectively, for compounds **19–21**. These values are different from 4.60 and 78.41 ppm, which correspond to CHOH of the starting alcohol **18**. Other signals related to the side chain moiety were also easily observed, particularly the carbons and protons of carbons bonded to the nitrogen atom of the amide group ($CONCH_3$ and $CONCH_2$). For compound **19** (as an example), these protons appear as two singlets (2.96 ppm and 2.98 ppm;

NCH_3) and two triplets (3.26 ppm and 3.40 ppm; NCH_2); the carbons appear as two peaks for NCH_3 (33.79 ppm and 35.65 ppm) and also two peaks for NCH_2 (47.85 ppm and 50.12 ppm). Duplication of the signal in ^1H and ^{13}C NMR spectroscopy is explained by the two conformations of the amide bond [41, 42] and was already observed for compounds with a similar side chain [25–27]. By mass spectrometry, thioether derivatives **19–21** do not show a molecular peak (M^+) (except compound **19**, the peak of which is very weak), but all compounds show an important and characteristic $M^+ - 135$ (HOPhCHEt) peak.

The NMR spectra of compounds **19** and **20** are very similar (except for the additional four protons in the 1.0–1.4 ppm area of compound **20**). These compounds thus have the same stereochemistry at their asymmetric centers and were designated diastereoisomer A (a racemic mixture of enantiomers). The other diastereoisomer was designated diastereoisomer B, or compound **21** (when $n = 10$ for side chain). The main ^1H NMR differences between the two diastereoisomers A **20** and B **21** were: (a) the triplet of the ethyl group ($CH_2\underline{CH_3}$) appears at 0.57 and 0.70 ppm, respectively; (b) the signal of $\underline{CH}$Et appears at 2.71 and 2.80 ppm; and (c) the doublets of aromatic protons appear at 6.81, 6.84, 7.01, 7.13, and 6.63, 6.65, 6.75, 6.88 ppm, respectively. The CH_2 signals of the ethyl group were also different, but unfortunately they were located in two multiplets. Other protons surrounding the chiral center ($\underline{CH}S\underline{CH_2}$) were slightly affected. By ^{13}C NMR no different chemical shifts were observed between the two diastereoisomers A and B. Despite the fact that a correlation between NMR data and stereochemistry was not possible, diastereoisomer A were assigned to compounds **19** and **20** and diastereoisomer B to compound **21** according to biological results (see Discussion).

Synthesis of nonsteroidal thioethers with an aryl side chain: 24–27, 35–37 (Fig. 4)

The coupling (Fig. 4) of three types of racemic-activated alcohol **18**, **22** [32], **23** [32] and four kinds of mercapto phenyl derivatives (thiophenol, HSPh; *p*-hydroxy thiophenol, HSPhOH; *p*-bromo-thiophenol, HSPhBr; *p*-terbutyldimethylsilyloxy thiophenol. HSPhOTBDMS) in the presence of zinc iodide was performed according to the methods described above and produced phenyl thioether derivatives in good yields (see Table 1 for conditions and yields of compounds **24–30**). The formation of new compounds was evidenced by a suitable molecular peak (M^+) in mass spectrometry and by additional signals for a new aryl group and characteristic signal of CHS arrangement (4.11–4.50 ppm in ^1H NMR and 53.32–56.53 ppm in ^{13}C NMR) in NMR spectroscopy. Similar to the previous section, this coupling reaction yielded a mixture of diastereoisomers A (**29**) and B (**30**) (each of them being a racemic mixture of enantiomers) (**29** and **30**) when the ethyl group (alcohol **18**) was used as starting material. Compounds **24–27** were used after final purification for biological analysis and compounds **27–29** for the synthesis of dimethyl amino derivatives **35–37**. Introduction of the dimethyl amino ethyl group (synthesis of compounds **33–35**) was achieved in two ways. The first method was used when dimethoxy thioether **27** was the starting phenol. In this case, we dissolved the phenol in DMF with K_2CO_3, 18-crown-6 and 2-dimethyl amino ethyl chloride hydrochloride. The mixture was stirred for 12 h at 25°C and refluxed for an additional 1 h, giving dimethoxy amino compound **35** in good yield (71%). The second method was used when di-THP thioethers **31**, **32** (obtained from TBDMS-derivatives **28**, **29**) were the starting phenols. According to the procedure of McCague *et al.* [43], we dissolved phenol in dry DMF and used sodium hydride to form an anion. At this time, 2-dimethyl aminoethyl chloride was added yielding di-THP amino derivatives **33**, **34**. Introduction of the dimethyl amino ethyl group was easily evidenced by IR as the disappearance of the phenol band, by NMR as the appearance of $OCH_2CH_2N(CH_3)_2$ signals (4.05 (t, 2H), 2.64 (t, 2H), 2.25 (s, 6H) (see compound **35** as example), and by mass spectrometry (suitable molecular peak). The two THP derivatives **33**, **34** were hydrolyzed (*p*-TSA, MeOH) to give the phenolic compounds **36**, **37**. However, for compounds with ethyl group **34**, we observed after hydrolysis a partial racemization of the chiral center, resulting in a mixture of two diastereoisomers A and B (65:35). After final purification, these compounds were used for biological evaluation.

Synthesis of thioether estradiol derivatives with alkyl-amide side chain (43–45 and 47) (Fig. 5)

Estrone (**38**) was transformed into 3-acetoxy estrone (**39**) using a mixture of acetic anhydride and pyridine (50/50). Oxidation of 3-acetoxy estrone (**39**) at the benzylic position (C-6) was achieved by CrO_3 and 3,5-dimethyl pyrazole in CH_2Cl_2 according to the method described by Takagi *et al.* [35]. The synthesis of 6-hydroxy estradiol (**41**) was performed by $LiAlH_4$ reduction of 3-acetoxy 6-oxo estrone (**40**). The resulting mixture of 6α/β-hydroxy estradiol (**41**) was used for coupling with two types of side chains (mercapto ester, $HS(CH_2)_2COOCH_3$ and mercapto amide; $HS(CH_2)_nCONBuMe$) according to the methods described above. Four racemics 6-thioether estradiol derivatives **42–45** were obtained in moderate yields (Table 1).

The new steroid compounds have a very characteristic signal displacement in ^1H NMR. The 6-CH signal was located at 4.70 ppm for alcohol **41**; the same signal was shielded for 6-thio derivatives (4.10 and 4.17 ppm for **42**, 4.05 and 4.13 ppm for **43**, 4.08 and 4.14 ppm for **44**, 4.01 and 4.05 ppm for **45**). The general characteristics of the amide side chain already discussed were also observed in this series of steroidal derivatives. In

Table 2. NMR chemical shift (δ) observed for three types of protons and used for identification of the C-6 side chain orientation (steroidal thioether series)

	δ (ppm)						
Proton localization	**41** Starting alcohol (CD_3OD)	**42** α/β* ($CDCl_3$)	**43** α/β* ($CDCl_3$)	**44** α/β* ($CDCl_3$)	**45A** α/β* ($CDCl_3$)	**45B** α* ($CDCl_3$)	**47** α/β* ($CDCl_3$)
18-CH_3 (s)	0.77	0.74	0.75	0.75	0.74	0.75	0.74
		0.80	0.81	0.82	0.82	—	0.82
6-CH (t_{app})	4.70	4.10	4.05	4.08	4.01	4.04	4.05
(d_{app})		4.17	4.13	4.14	4.05	—	4.10
4-CH (d)	6.97	6.81	6.85	6.87	6.85	—	6.84
(d)		7.12	7.20	7.18	7.18	7.19	7.16

*Side chain orientation in the 6-position.

IR, ester and amide bands were observed for compounds **42** and **43–45**, respectively. Moreover, by mass spectrometry, we observed the molecular peak (M^+) for all compounds. As mechanistically anticipated and chemically observed, the coupling reaction of 6-hydroxy estradiol with the mercapto side chain yields a C-6 racemic mixture ($\alpha:\beta$/50:50). In fact, after formation of C-6 carbocation (Sn1 process) the mercapto chain can attack each side of the steroid, leading to a racemic mixture. Attribution ($\alpha:\beta$) of the C-6 isomeric mixture was achieved by ^{1}H NMR spectroscopy using the chemical shifts (δ) of 18-CH_3 group, 6-CH proton and 4-CH proton (Table 2). For the methyl group, two signals were observed for the mixture of α/β diastereoisomers (0.74–0.75 ppm and 0.80–0.82 ppm), one similar to the methyl signal of the starting alcohol **41** (0.77 ppm). We assigned this signal to the compound with 6α stereochemistry, as the side chain in the 6α-position cannot influence the methyl group with a β orientation. On the other hand, the compound with a more deshielded methyl signal was attributed to the 6β-configuration. In this case, the amido group of the side chain influenced the methyl group on the β-face of the steroid. For the proton in the 6-position, two signals were observed (4.01–4.10 ppm as apparent triplet and 4.05–4.17 ppm as doublet). According to computer evaluation of angles, we estimated that the proton in the 6β-position (6α-side chain) would have two large coupling constants with the vicinal (C-7) protons and give a doublet or an apparent triplet. The other diastereoisomeric proton in the 6α-position (6β-side chain) was expected to have a smaller coupling constant and give an apparent doublet. In fact, in an additional 2D NMR experiment (J resolve) [44]* performed with each separated isomer of compound **43**, the 6α-side chain derivative was a doublet of doublet ($J_1 = 6$ Hz and $J_2 = 11$ Hz); the other isomer, a 6β-side chain derivative, was also a doublet of doublet but with smaller coupling constants ($J_1 = 3$ Hz and $J_2 = 6$ Hz). Finally, for the aromatic proton in the 4-position, two signals were also observed: the first at 6.81–6.87 ppm and the other at 7.12–7.20 ppm. The deshielded signal was attributed to the compound with an α-side chain. In this configuration (α), the amide group seems able to affect the aromatic proton but not in the other configuration (β). During final purification by chromatography of compounds **42–45**, the α-isomer was enriched and biological analysis performed using these mixtures (Table 5). The proportions of isomers were evaluated by HPLC (Water Associates, Milford, MA) with a reverse-phase column (Nova-Pak, C-18, 4 μm, 0.5 cm × 10 cm) and by NMR (18-CH_3 signal).

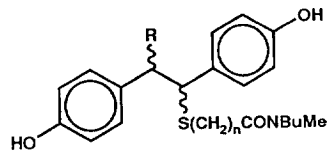
The thioether with a short alkylamide side chain **47** was synthesized from ester derivative **42**, which was previously obtained by the general coupling strategy (Fig. 5). The methyl ester was hydrolyzed to the corresponding carboxylic acid (KOH, H_2O, MeOH), which, without further purification, was transformed to methyl butyl amide. During this process, the free phenolic group was transformed to carbonate derivative **46**. This compound was formed by reaction of the phenol group and isobutylchloroformate used for the amide preparation. Compound **46**, however, was easily hydrolyzed (K_2CO_3, H_2O, MeOH) to corresponding phenolic derivative **47**. We observed the characteristic signal displacements in ^{1}H NMR, amide band in IR, and molecular peak in mass spectrometry for this compound.

Biological Activity

To assess the biological activity of the new compounds, two different assays were used: the relative binding affinity (RBA) for the estrogen receptor and the *in vitro* estrogenic or antiestrogenic activity, as revealed by the effect of tested compounds on the proliferation of estrogen-sensitive ZR-75-1 human breast cancer cells. The RBA was determined *in vitro* using cytosol from immature rat uterus. Using the IC_{50} values of displacement of the tested compounds and E_2, the RBA was calculated by arbitrarily taking the binding of E_2 as

*The 2D J resolve spectrum was obtained using the standard pulse sequence. The experiment was performed on a Bruker ACF-300 spectrometer operating at 300.13 MHz. The spectrum was acquired with 128 × 1 K data points [44].

*Table 3. Biological activities of nonsteroidal thioethers with alkylamide side chain (**19**, **20**, **21**, **48**, **49**)*



No.	R	n	ER-binding (%)* RBA	10 μM	ZR-75-1 cells† Estrogenic activity (%)‡	Antiproliferative activity (%)§	Antiestrogenic activity (%)‖
48¶	H	7	0.01	—	None	28 ± 2	44 ± 6
49¶	H	10	0.01	—	None	41 ± 3	80 ± 7
19	CH_3CH_2	7 (A)	0.41	57	4 ± 4	None	87 ± 1
20	CH_3CH_2	10 (A)	1.1	73	None	11 ± 4	89 ± 2
21	CH_3CH_2	10 (B)	—	17	33 ± 3	None	62 ± 3
EM-139(**10**)			1.2	90	None	14 ± 3	106 ± 6

*Binding affinity for rat uterine cytosolic estrogen receptor; RBA, relative to estradiol (E_2 = 100%) or at a concentration of 10 μM (E_2 = 100%).

†Assays in ZR-75-1 cells were performed in triplicate. Compounds were tested at a concentration of 1 μM. See experimental section for cell growth conditions and equations.

‡Stimulation of cell proliferation or estrogen activity.

§Inhibition of basal cell proliferation or antiproliferative activity.

‖Inhibition of E_2 (0.1 nM)-induced cell proliferation or antiestrogenic activity.

¶These results were obtained from another experiment (see reference [32]).

100%. Binding to the estrogen receptor was also expressed in percentage of displacement of [^{3}H]E_2 at one dose (10 μM) of the tested compounds. For the estrogenic and antiestrogenic assay, we measured the ability of the compounds to stimulate or inhibit the proliferation of ZR-75-1 cells and to inhibit E_2 (0.1 nM)-stimulated proliferation of the same cells at a concentration of 1 μM.

Nonsteroidal thioethers with an alkylamide side chain

Table 3 illustrates that compound **20** gave a somewhat higher RBA value than compound **19** (with a shorter alkylamide side chain) and compounds **48**, **49** (without ethyl group) [32]. For the same side chain length, diastereoisomer A (**20**) had a better binding affinity than diastereoisomer B (**21**), at respective values of 73 and 17% displacement of [^{3}H]E_2. The best compound of this series, compound **20**, had a RBA value (1.1%) similar to the RBA of EM-139 (**10**) (1.2%). At the concentration used, a significant estrogenic activity was observed only for compound **21** (diastereoisomer B). The estrogenic effect of compound **19** was not significant. Compounds **20**, **48**, and **49** produced an inhibition of basal cell proliferation (antiproliferative activity) similar to EM-139. Under similar cell growth conditions, estrogens stimulated the proliferation of these estrogen-sensitive cells, whereas the pure antiestrogen EM-139 (**10**) had no effect.

The ability of the new compounds to inhibit E_2 (0.1 nM)-induced stimulation of ZR-75-1 cell proliferation was measured and reported as antiestrogenic activity. In the screening assay, all tested compounds inhibited the E_2 stimulation of cell proliferation by 44 to 89%. At the same concentration, EM-139 provoked total inhibition of the E_2 stimulation of ZR-75-1 cell proliferation. Figure 6 compares the effect of increasing concentrations of four compounds of Table 3 (**19**, **20**, **21** and **49**) with that of pure antiestrogen EM-139 (**10**). In agreement with the data from the ZR-75-1 screening assay (Table 3), no estrogenic activity was observed for compounds **19**, **20**, **49** and EM-139, whereas in higher concentrations, compound **21** showed a weak estrogenic activity. The steroidal antiestrogen EM-139 (**10**) exerted a more potent inhibitory effect on E_2-stimulated cell proliferation (IC_{50} value of 25 nM) compared with the nonsteroidal derivatives **49**, **19**, and **20** (IC_{50} values of 700, 380 and 250 nM, respectively). Compound **21**, on the other hand, showed no significant inhibition of E_2-stimulated proliferation. The effect of the ethyl group was clearly observed when we compared the IC_{50} values of **20** and **49**. In this case, compound **20** with the ethyl group inhibited by 3-fold E_2-stimulated cell proliferation compared with compound **49** without the ethyl group. We also observed that inhibitory activity decreased slightly with the shortening of the side chain, as suggested by the respective IC_{50} values of compounds **19** and **20**. Compound **20**, which has a long alkylamide side chain similar to EM-139 (**10**) or ICI 164384 (**9**), in addition to an ethyl group and a stereochemistry of asymmetric centers related to steroidal nucleus (C-8, C-9), showed somewhat better antiestrogenic activity. This result is

also in agreement with the fact that compound **20** has the highest relative binding affinity.

Nonsteroidal thioethers with an aryl side chain

Compounds listed in Table 4 showed a binding affinity for the estrogen receptor with percentages of inhibition of [^{3}H]E_2 binding ranging from 39 to 83% at 10 μM. Compounds **24**, **26**, and **37** (80, 77 and 83%) had a percentage of displacement similar to that of EM-139 (**10**) (90%) at 10 μM, and the other compounds (**25**, **27**, **35**, **36**) possessed a binding affinity lower than EM-139 (**10**). Compound **37**, with an ethyl group at α-position of the side chain, had a better binding affinity than compound **36** without the ethyl group (83 and 49%, respectively). In ZR-75-1 cells, all compounds (except **35**) stimulated cell proliferation; values ranged from 29 to 216% at a concentration of 1 μM, thus indicating their estrogenic activity. For compound **35**, no estrogenic activity was observed, this result being related to the blockade of the phenolic group by a methoxyl group. A free phenolic group is recognized as a very important characteristic for *in vitro* binding to the estrogen receptor. Interestingly, compound **27**, with two methoxy groups and one free phenolic group, is estrogenic (56%). Compound **37** with an ethyl group is more estrogenic than compound **36** without the ethyl group (216% and 29%, respectively). As already noted for compounds **20** and **49** of the first series, the introduction of an ethyl group improved the binding on the estrogen receptor and, consequently, the estrogenic or antiestrogenic activity. Under these screening conditions, EM-139 (**10**) and OH-tamoxifen (**2**) showed no estrogenic activity; significant inhibition of basal cell proliferation was observed only for EM-139.

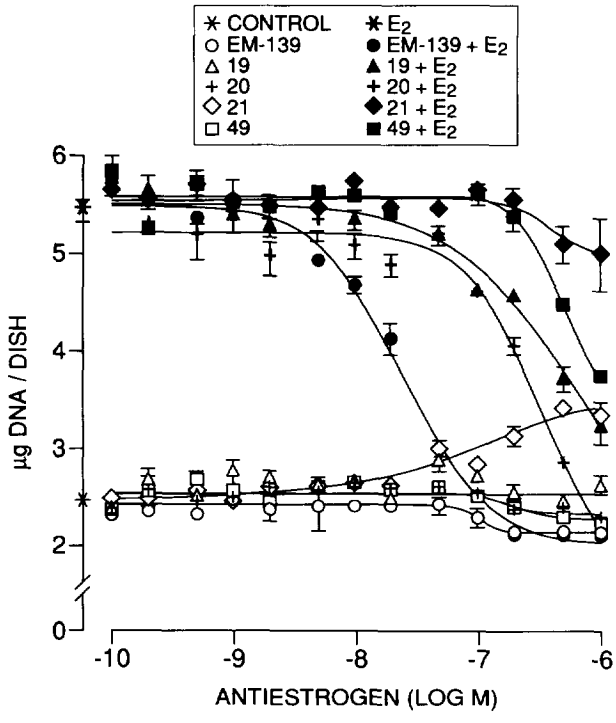


Fig. 6. Comparison of the effects of compounds 19, 20, 21, and 49 with those of the pure antiestrogen EM-139 on basal (open symbols) and E_2-induced cell proliferation (closed symbols) in ZR-75-1 human breast cancer cells. The IC_{50} values obtained in the presence of E_2 were 25, 250, 380 and 700 nM, respectively, for compounds EM-139, 20, 19, and 49. Data obtained in the absence of the indicated antiestrogen are indicated on the y axis. Three days after plating, cells were incubated for 9 days with the indicated concentrations of antiestrogens in the presence or absence of 0.1 nM E_2. Media were changed every second day. Results are expressed as mean ± SEM of triplicate dishes. When SEM overlaps with the symbol, only the symbol is shown.

Steroidal thioethers with an alkylamide side chain

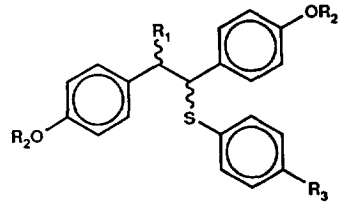
In the third series of compounds (Table 5), an alkylamide side chain was introduced at the 6-position of the estradiol nucleus by a thioether link. These compounds were related to the ICI 164384-structure, differing in their side chain position (6 instead of 7) and by the presence of a sulfur atom at the junction of the steroidal skeleton and side chain. Despite this structural similarity, the biological results were very different (Table 5). In fact, these compounds (**43–45** and **47**) were fully estrogenic, as revealed by their potent proliferative effect in ZR-75-1 cells (82 to 110% at 1 μM). Moreover, similar estrogenic effects (108 and 110%) were obtained for two preparations of compound **45** which possess two different 6α and 6β side chain orientations (36:64 and 100:0). No antiproliferative activity was observed for these compounds. Inversely, under these screening conditions, EM-139 (**10**) showed no estrogenic activity and a significant antiproliferative activity (14%).

DISCUSSION

The synthesis of 14 new compounds was achieved with the aim of improving the biological activity of the known antiestrogen tamoxifen (**1**) and to simplify the long, arduous chemical synthesis of the pure antiestrogen ICI 164384 (**9**) or EM-139 (**10**). Thus, using the efficient reaction of thioether formation, three types of compounds were easily synthesized: (i) nonsteroidal derivatives with an alkylamide side chain (Table 3); (ii) nonsteroidal derivatives with an aryl side chain (Table 4); and (iii) steroidal derivatives with an alkylamide side chain at the 6-position (Table 5).

The results of the screening tests (Tables 3–5) show that of the three types of compounds studied, only the nonsteroidal derivatives that have an alkylamide side chain possess substantial antiestrogenic activity. The two other categories of compounds were rather deceptive. In the steroidal series (Table 5), displacement of the alkylamide side chain from the 7- to the 6-position produced compounds with chemical characteristics similar to ICI 164384 (**9**) or EM-139 (**10**); unfortunately, these new compounds were fully estrogenic.

Table 4. Biological activities of nonsteroidal thioethers with an aryl side chain (24–27, 35–37)

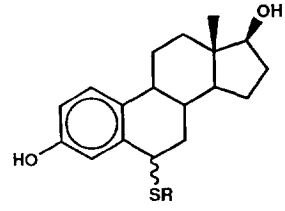


No.	R_1	R_2	R_3	ER-binding (%)*	ZR-75-1 cells† Estrogenic activity (%)‡	ZR-75-1 cells† Antiproliferative activity (%)§
24	H	H	H	80	49 ± 4	None
25	H	H	Br	39	128 ± 9	None
26	H	H	OH	77	74 ± 7	None
27	H	CH_3	OH	49	56 ± 5	None
35	H	CH_3	$O(CH_2)_2N(CH_3)_2$	43	None	6 ± 3
36	H	H	$O(CH_2)_2N(CH_3)_2$	49	29 ± 10	None
37‖	CH_3CH_2	H	$O(CH_2)_2N(CH_3)_2$	83	216 ± 6	None
OH-TAM (2)			—	—	None	None
EM-139 (**10**)			—	90	None	14 ± 3

*Binding affinity for rat uterine cytosolic estrogen receptor at a concentration of 10 μM ($E_2 = 100\%$).
†Assays in ZR-75-1 cells were performed in triplicate. Compounds were tested at a concentration of 1 μM. See experimental section for cell growth conditions and equations.
‡Stimulation of cell proliferation or estrogenic activity.
§Inhibition of unstimulated cell proliferation or antiproliferative activity.
‖As a mixture of diastereoisomers in 65:35 proportion.

This lack of antiestrogenic activity can be explained by the different orientation of the side chain at the 6-position: pseudoaxial (6α) or pseudoequatorial (6β). In fact, pure steroidal antiestrogens, such as ICI 164384 (**9**), EM-139 (**10**), and RU 51625 (**13**), have their alkylamide side chain in the true axial orientation (7α

Table 5. Biological activities of steroidal thiothers (43–45 and 47)



No.	R	C-6 (α:β)*	ZR-75-1 cells† Estrogenic activity (%)	ZR-75-1 cells† Antiproliferative activity (%)§
47	$(CH_2)_2CONBuMe$	35:65	107 ± 7	None
43	$(CH_2)_5CONBuMe$	49:51	91 ± 4	None
44	$(CH_2)_7CONBuMe$	33:67	82 ± 6	None
45A	$(CH_2)_{10}CONBuMe$	36:64	108 ± 9	None
45B	$(CH_2)_{10}CONBuMe$	100:0	110 ± 8	None
EM-139 (**10**)			None	14 ± 3

*The proportion (α:β) was evaluated by HPLC (Waters Associates, Milford, MA) with reverse-phase column (C-18, 4 μM, 0.5 × 10 cm) using an appropriate mixture of CH_3CN, MeOH, H_2O as eluent.
†ZR-75-1 cell assays were performed in triplicate. Compounds were tested at a concentration of 1 μM. See experimental section for cell growth conditions and equations.
‡Stimulation of cell proliferation or estrogenic activity.
§Inhibition of unstimulated cell proliferation or antiproliferative activity.

or 11β). As proposed by Jordan and Koch [45], the side chain in the 7α or 11β-position is located in the same spatial area (if a rotation of 180° is done in the steroidal plane), an area that seems very important for antiestrogenic activity. With the new steroidal derivatives, the spatial area occupied by the side chain in the 6α- or 6β-position was not sufficiently related to this important area (drieding and computer model), and the side chain could not play its potential antiestrogenic function.

In the nonsteroidal series of compounds with an aryl side chain (Table 4), an estrogenic activity was observed for all thioether compounds except the dimethoxy derivative with arylamine side chain. However, it could be expected that this compound would be estrogenic after *in vivo* cleavage of the methoxy group; in fact, the phenolic analog was estrogenic. Thus, no improvement in biological activity could be obtained, despite the fact that these compounds are nonisomerizable (no double bond) analogs of tamoxifen. In fact, the bond isomerization was generally reported as an explanation for the mixed activity of tamoxifen derivatives (the Z isomer is antiestrogenic, and the E isomer is estrogenic); however, our results show that blockade of isomerization cannot be done without maintaining molecular planarity.

The introduction of an alkylamide side chain on the diaryl ethanol nucleus produces better antiestrogenic activity than use of the dimethylaminoethoxyaryl side chain. In fact, the new nonsteroidal thioethers related to ICI 164384 (**9**) or EM-139 (**10**) (Table 3) possess the same biological profile as pure antiestrogens: no estrogenic activity, a small inhibition of basal cell proliferation, and a good inhibition of E_2-induced cell proliferation (antiestrogenic activity) in the ZR-75-1 human breast cancer line. Slightly better results were achieved for compounds having a side chain length similar to ICI 164384 (**9**) or EM-139 (**10**). Moreover, binding affinity was improved when an ethyl group was added on the diaryl ethanol nucleus (diaryl butanol nucleus). For this nucleus, however, the chemical synthesis led to the formation of two diastereoisomers A and B (each of them being a racemic mixture of two enantiomers) that have very different biological activity. The diastereoisomer (A) possesses an RBA identical to EM-139 (1.1 and 1.2%, respectively) and a significant antiestrogenic activity (IC_{50} 250 nM), whereas the other diastereoisomer (B) possesses a very weak binding affinity and no antiestrogenic activity. Moreover, this diastereoisomer has weak estrogenic activity. According to biological activity, we assigned the configuration S,S and R,R (like **20**) to diastereoisomer A and the configuration S,R and R,S (like **21**) to diastereoisomer B. The configuration S,S and R,R of diastereoisomer A is similar to the 8β-H and 9α-H configuration of the steroidal nucleus, but configurations S,R and R,S are different. Thus, compounds with a S,S and R,R configuration of pseudo 8- and 9-carbon (diastereoisomer A) superpose the steroidal skeleton better than diastereoisomer B does (S,R and R,S configuration). For this reason, the spatial orientation of the pseudo 7α- or 11β-alkylamide side chain of diastereoisomer A is closely related to the spatial orientation of the 7α- or 11β-alkylamide side chain of a pure steroidal antiestrogen, thus producing antiestrogenic activity. In agreement with the good analogy of nonsteroidal thioethers with an alkylamide side chain and the pure steroidal antiestrogens, the best derivative of the series **20** (Table 3) was about 10-fold less antiestrogenic than EM-139 (**10**). However, the ease of synthesis of these compounds compared with the long synthesis of steroidal antiestrogen, encourages us to pursue work on these thioether derivatives while focusing on the refinement of the chemical structure for improved antiestrogenic activity.

Acknowledgements—We would like to thank Karine Andryancyzk and Diane Michaud for their skilful technical assistance, Charles Lévesque for HPLC-purity evaluation of tested compounds, and Patricia Dionne for 2D NMR-experiments (J resolve). A constructive remark of one reviewer was greatly appreciated. We also thank the Medical Research Council of Canada (MRC group in molecular endocrinology), the Fonds de la Recherche en Santé du Québec (FRSQ), and Endorecherche for their financial support.

REFERENCES

1. Silverberg E., Squires T. S. and Boring C. C.: Cancer statistics. *CA—Cancer J. Clin.* **43**, (1993) 7–26.
2. Jordan V. C. and Murphy C. S.: Endocrine pharmacology of antiestrogens as antitumor agents. *Endocrine Rev.* **11** (1990) 578–610.
3. Gottardis M. M. and Jordan V. C.: Development of tamoxifen-stimulated growth of MCF-7 tumors in athymic mice after long-term antiestrogen. *Cancer Res.* **48**, 5183–5187.
4. Sutherland R. L. and Murphy L. C.: Mechanisms of oestrogen antagonism by nonsteroidal antioestrogens. *Molec. Cell. Endocr.* **25** (1982) 5–23.
5. Gottardis M. M., Robinson S. P., Satyaswaroop P. G. and Jordan V. C.: Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. *Cancer Res.* **48** (1988) 812–815.
6. Wakeling A. E.: Therapeutic potential of pure antiestrogens in the treatment of breast cancer. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 771–775.
7. Poulin R., Mérand Y., Poirier D., Lévesque C., Dufour J. M. and Labrie F.: Antiestrogenic properties of keoxifene, trans-4-hydroxytamoxifen and ICI 164384, a new steroidal antiestrogen, in ZR-75-1 human breast cancer cells. *Breast Cancer Res. Treat.* **14** (1989) 65–76.
8. Murphy C. S., Parker C. J., McCague R. and Jordan V. C.: Structure–activity relationships of nonisomerizable derivatives of tamoxifen: importance of hydroxyl group and side chain position for biological activity. *Molec. Pharmac.* **39** (1991) 421–428.
9. Chander S. K., McCague R., Luqmani Y., Newton C., Dowsett M., Jarman M. and Coombes R. C.: Pyrrolidino-4-iodotamoxifen and 4-iodotamoxifen, new analogues of the antiestrogen tamoxifen for the treatment of breast cancer. *Cancer Res.* **51** (1991) 5851–5858.
10. Jones C. D., Suarez T., Massey E. H., Black L. J. and Tinsley C.: Synthesis and antiestrogenic activity of [3,4-dihydro-2-(4-methoxyphenyl)-1-napthalenyl] [4-[2-(1-pyrrolidinyl)ethoxyl]-phenyl] methanone, methanesulfonic acid salt. *J. Med. Chem.* **22** (1979) 962–966.
11. Black L. J. and Goode R. L.: Uterine bioassay of tamoxifen, trioxifene and a new estrogen antagonist (LY 117018) in rats and mice. *Life Sci.* **26** (1980) 1453–1458.
12. Jones C. D., Jevniker M. G., Pike A. J., Peters M. K., Black

L. J., Thompson A. R., Falcone T. F. and Clemens J. A.: Antiestrogens. 2′-structure–activity studies in a series of 3-aroyl-2-arylbenzo[b]thien-3-yl [4-[2-(1-piperidinyl)ethoxy]-phenyl]methanone hydrochloride (LY 156758), a remarkably effective estrogen antagonist with only minimal intrinsic estrogenicity. *J. Med. Chem.* **27** (1984) 1057–1066.
13. Jones C. D., Blaszczak L. C., Goettel M. E., Suarez T., Crowell T. A., Mabry T. E., Ruenitz P. C. and Srivatsan V.: Estrogen receptor affinities and antiproliferative effects in MCF-7 cells of phenolic analogues of trioxifen. *J. Med. Chem.* **35** (1992) 931–938.
14. Jordan V. C. and Gosden B.: Inhibition of uterotrophic activity of estrogen by the short acting antiestrogen LY-117018. *Endocrinology* **113** (1983) 463–468.
15. Clemens J. A., Bennett D. R., Black L. J. and Jones C. D.: Effect of a new antiestrogen, keoxifen (LY 156758) on growth of carcinogen-induced mammary tumors and on LH and prolactin levels. *Life Sci.* **32** (1983) 2869–2875.
16. Gottardis M. M. and Jordan V. C.: The antitumor actions of keoxifene and tamoxifen in the *N*-nitrosomethylurea-induced rat mammary carcinoma model. *Cancer Res.* **47** (1987) 4020–4024.
17. Wakeling A. E. and Bowler J.: Steroidal pure antiestrogens. *J. Endocr.* **112** (1987) R7–R10.
18. Wakeling A. E. and Bowler J.: Biology and mode of action of pure antioestrogens. *J. Steroid Biochem.* **30** (1988) 141–147.
19. Bowler J., Lilley T. J., Pittam J. D. and Wakeling A. E.: Novel steroidal pure antiestrogens. *Steroids* **54** (1989) 71–79.
20. Pasqualini J. R. and Gelly C.: Biological response of the anti-estrogen ICI 164384 in human hormone-dependent and hormone-independent mammary cancer cell lines. *Cancer Lett.* **50** (1990) 133–139.
21. Lévesque C., Mérand Y., Dufour J. M., Labrie C. and Labrie F.: Synthesis and biological activity of new halo-steroidal antiestrogens. *J. Med. Chem.* **34** (1991) 1624–1630.
22. Labrie C., Martel C., Dufour J. M., Lévesque C., Mérand Y. and Labrie F.: Novel compounds inhibit estrogen formation and action. *Cancer Res.* **52** (1992) 610–615.
23. Nédelec L., Nique F., Bouton M. M. and Van De Velde P.: Synthesis and estrogenic/antiestrogenic activities of a series of 7α-aryl-estradiols and comparison with 11β-aryl-estradiols. *Proc. 8th Int. Congress on Hormonal Steroids*, The Hague, The Netherlands (1990) Abst. No. 302.
24. Nédelec L., Bouton M. M., Nique F., Teutsch G., Van De Velde P. and Philibert D.: 11β-amino-alkoxyphenylestradiols, a new series of potent antiestrogens. *9th Int. Symp. J. Steroid Biochem*, Las Palmas, Spain (1989) Abst. No. 34P.
25. Poirier D., Mérand Y. and Labrie F.: Synthesis of 17β-estradiol derivatives with *N*-butyl, *N*-methyl alkylamide side chain at position 15. *Tetrahedron* **47** (1991) 7751–7766.
26. Poirier D. and Labrie F.: Synthesis of estradiol derivatives with alkylamide side chain in 17α- or 15α-position. *73th CIC Congress*, Halifax, Canada (1990) Abst. No. 835.
27. Poirier D., Labrie C., Mérand Y. and Labrie F.: Synthesis and biological activity of 17α-alkynylamide derivatives of estradiol. *J. Steroid Biochem. Molec. Biol.* **38** (1991) 759–774.
28. Claussner A., Nédelec L., Nique F., Philibert D., Teutsch G. and Van De Velde P.: 11β-amidoalkyl estradiols, a new series of pure antiestrogens. *J. Steroid. Biochem. Molec. Biol.* **41** (1992) 609–614.
29. Wakeling A. E., Dukes M. and Bowler J.: A potent specific pure antiestrogen with clinical potential. *Cancer Res.* **51** (1991) 3867–3873.
30. Von Angerer E., Knebel N., Kager M. and Ganss B.: 1-(aminoalkyl)-2-phenylindoles as novel pure estrogen antagonists. *J. Med. Chem.* **33** (1990) 2635–2640.
31. Nishino Y., Schneider M. R., Michna H. and von Angerer E.: Pharmacological characterization of a novel oestrogen antagonist, ZK-119010 in rats and mice. *J. Endocr.* **130** (1991) 409–414.
32. Poirier D., Auger S., Mérand Y. and Labrie F.: Synthesis and antiestrogenic activity of diaryl thioether derivatives. *J. Med. Chem.* **37** (1994) 1115–1125.
33. Buu-Hoi N. P., Sy M. and Xuong N. D.: Sur la réaction de Pfitzinger avec les cétones phénoliques. *Bull. Soc. Chim. Fr.* **23** (1956) 629–632.
34. Paquet A. and Layne D. S.: Reactivity of the thallium (I) salts of 3-hydroxy-1,3,5 (10)-estratrien-17-one and 1,3,5 (10)-estratriene-3,17β-diol. Preparation of 17-oxoestra-1,3,5 (10)-trien-3-yl 2′-acetamido-3′,4′,6′-tri-O-acetyl-2′-deoxy-β-D-glugopyranoside. *Can. J. Chem.* **51** (1973) 3855–3859.
35. Takagi H., Komatsu K. and Yoshizawa I.: Synthesis and mechanism of hydrolysis of estrogen-6-sulfates: model compounds for demonstrating the carcinogenesis of estrogen. *Steroids* **56** (1991) 173–179.
36. Asselin J. and Labrie F.: Effects of estradiol and prolactin on steroid receptor levels in 7,12-dimethylbenz(a)anthracene-induced mammary tumors and uterus in the rat. *J. Steroid Biochem.* **9** (1978) 1079–1082.
37. Fiszer-Szafarz B., Skafarz D. and Guevara De Murillo A. A general, fast and sensitive micromethod for DNA determination: application to rat mouse liver, rat hepatoma, human leukocytes, chicken fibroblasts and yeast cells. *Analyt. Biochem.* **110** (1981) 165–170.
38. Simard J., Dauvois S., Haagensen D. E., Lévesques C., Mérand Y. and Labrie F.: Regulation of progesterone binding breast cyst protein GCDFP-24 secretion by estrogens and androgen in human breast cancer cell: a new marker of steroid action in breast cancer. *Endocrinology* **126** (1990) 3223–3231.
39. Guindon Y., Frenette R., Fortin R. and Rokach J.: Direct synthesis of thioethers from thiols and alcohols. *J. Org. Chem.* **48** (1983) 1357–1359.
40. Corey E. J., Niwa H. and Knolle J.: Total synthesis of (S)-12-hydroxy-5,8,14-cis,-10 *trans*-eicosatetraenoic acid (Samuelsson's HETE). *J. Am. Chem. Soc.* **100** (1978) 1942–1943.
41. Dionne P., Singh S. M. and Labrie F.: ^{1}H and ^{13}C nuclear magnetic resonance assignments and stereochemistry of *N*-n-butyl-*N*-methyl-11-(16′α-chloro-3′,17′β- and 17′α-dihydroxy-estra-1′,3′,5′(10′)-trien-7′α-yl) undecanamide. *Steroids* **59** (1994) 493–497.
42. Levy G. C. and Nelson G. L.: *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*. Wiley-Interscience, Toronto (1972) pp. 120–123.
43. McCague R., Leclercq G., Legros N., Goodman J., Blackburn G. M., Jarman M. and Foster A. B.: Derivatives of tamoxifen. Dependence of antiestrogenicity on the 4-substituent. *J. Med. Chem.* **32** (1989) 2527–2533.
44. Two-dimensional NMR spectroscopy (Applications for chemists and biochemists). In *Methods in Stereochemical Analysis* (Edited by W. R. Croasmun and R. M. K. Carlston). VCH Publishers, NY (1987) Vol. 9 pp. 33, 281 and 404.
45. Jordan V. C. and Koch R.: Regulation of prolactin synthesis *in vitro* by estrogenic and antiestrogenic derivatives of estradiol and estrone. *Endocrinology* **124** (1989) 1717–1726.