**Table III—Sensitivity of Systems 1 and 2 to Structural Changes**

| Structural Change, $A_x \rightarrow A_y$ | Compounds | $\Delta_{12}$ |
|---|---|---|
| $\Delta_{12} \neq 0$ | | |
| Full Replacement of H | | |
| $-OH \rightarrow -OCH_3$ | X/IV | −0.21 |
| $-OH \rightarrow -OOCCH_3$ | VIII/VII | −0.69 |
| | XII/IV | −0.31 |
| $-NH_2 \rightarrow -N(CH_3)_2$ | VI/I | −0.28 |
| $-NH-OCCH_3 \rightarrow -N(CH_3)-OCCH_3$ | XIII/III | −0.15 |
| Chain Extension | | |
| $-OCH_3 \rightarrow -OC_2H_5$ | XI/X | +0.11 |
| $-COOCH_3 \rightarrow -COOC_2H_5$ | XIV/I | +0.32 |
| $-COOC_2H_5 \rightarrow -COO(CH_2)_2CH_3$ | XV/XIV | +0.16 |
| $-C_2H_5 \rightarrow -(CH_2)_2CH(CH_3)_2$ | XVIII/XVII | +1.12 |
| $\Delta_{12} \cong 0$ | | |
| Changes Which Preserve H | | |
| $-NH_2 \rightarrow -OH$ | VII/I | −0.09 |
| | XVI/XV | +0.05 |
| Hydrogen Replacement Plus Chain Extension | | |
| $-OH \rightarrow -OC_2H_5$ | XI/IV | 0.00 |

1); the chain extension $-OCH_3 \rightarrow -OC_2H_5$ (X→XI) generates a positive $\Delta_{12}$ (correlation 5). Obviously, the extent of the affinity of a functional group for a given medium is the result of a number of well-defined molecular interactions usually described as specific, nonspecific, solvophobic, *etc.* (8).

Because of the importance of the hydrophilic-lipophilic concept, considerable effort has been made to measure such properties from partition coefficient or solubility measurements (9, 10) and more recently from solvent effects on solubility (2, 3).

The results discussed here show that functional groups may exhibit different hydrophilic-lipophilic properties in different environments, *i.e.*, XVIII appears to be 16-fold more lipophilic than XVII in ethanol-water, but only ~1.2-fold more lipophilic in ethanol-cyclohexane. Thus, it is now clear that the order of hydrophilic strength of different functional groups in ethanol-water reported previously (3) should be quite different from the order of the same groups in ethanol-cyclohexane.

Just as Hammett pointed out the ambiguity of the concepts of acidity and basicity (11), the inherent ambiguity of the hydrophilic-lipophilic concept should be emphasized. However, Eq. 1 appears to be a useful tool to obtain information about the hydrophilic-lipophilic properties of different solvent media.

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# Synthesis and Evaluation of Novel *N*-Substituted *N'*-(3-Hydroxy-17-oxoestra-1,3,5(10)-trien-2- and -4-yl)thiourea Derivatives for Binding to the Estrogen Receptor and Cytotoxic Activity on MCF-7 Cells


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Received October 25, 1982, from the **Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Alexandria Egypt* and the ‡*Laboratoire de Cancérologie Mammaire, Service de Médecine, Institut Jules Bordet, 1000 Brussels, Belgium.* Accepted for publication October 26, 1983.

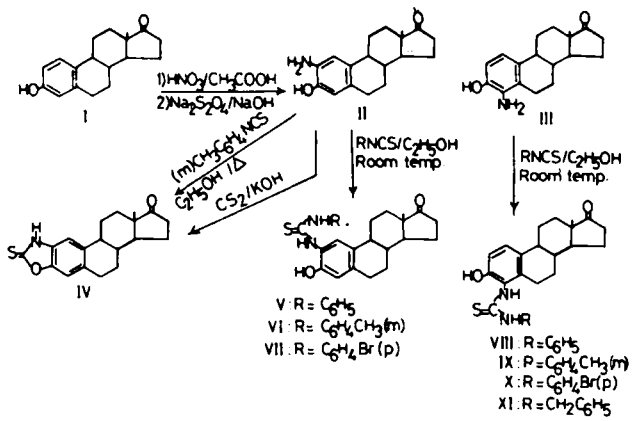


**Abstract** □ A novel series of estrone derivatives having a free 3-phenolic group with the 2- or 4-position substituted with a thiourea function was synthesized. None of the products showed significant binding to the estrogen receptor, and the cytotoxic activity on MCF-7 cells for VII and X was weak.



**Keyphrases** □ Estrone 2- and 4-thiourea derivatives—free phenolic group, synthesis, binding to the estrogen receptor, cytotoxic activity □ Steroidal thiourea derivatives—2- or 4-position of estrone, synthesis binding to the estrogen receptor, cytotoxic activity


Several types of compounds containing structural modifications of steroidal and nonsteroidal estrogens were synthesized during past years, in the hope of developing agents with a high binding affinity to the estrogen receptor (1, 2) and reduced estrogenic properties. These, in accordance with their capabilities in antagonizing the action of estradiol at the estrogen receptor (3), can be used as antiestrogens (4–9) or as cytotoxic agents with selective activity against the hormone-dependent tumor cells (10–13). In connection with an extensive program studying the effect of structural modifications on the biological activity of hormones, a variety of modified steroids were synthesized and tested for antiestrogenic (14), endocrinological (15–17), and anticancer (15, 17, 18) properties. As a supplemental investigation, a novel series of estrone thiourea derivatives (V–XI) were synthesized to check their binding to




*Scheme I*

the estrogen receptor and *in vitro* cytotoxic activity on the growth of cells from the estrogen receptor-positive breast cancer cell line MCF-7.

## RESULTS AND DISCUSSION

**Chemistry**—The synthesis of *N*-substituted *N'*-(3-hydroxy-17-oxoestra-1,3,5(10)-trien-2-yl)thioureas (V–VII) and the corresponding *N*-substituted *N'*-(3-hydroxy-17-oxoestra-1,3,5(10)-trien-4-yl)thioureas (VIII–XI) was accomplished as shown in Scheme I. The 2- and 4-aminoestrones (II and III, respectively), prepared by nitration of estrone (19) and reduction of the produced 2- and 4-mononitroestrones as previously reported (20), were allowed to react at room temperature with the equivalent amount of the selected arylisothiocyanate derivatives in ethanol or ethanol–acetone to give the estrone-2-thiourea derivatives (V–VII). The attempt to crystallize VI from boiling ethanol led to the elimination of *m*-toluidine and the formation of 2'-thio-17-oxoestra-1(10),4-dieno[2,3-*d*]oxazoline (IV). The same oxazoline derivative IV was obtained when equimolar amounts of 2-aminoestrone (II) and *m*-tolylisothiocyanate were heated in boiling ethanol for a short period or, as previously reported for other heat-sensitive thioureas (21), when VI was heated above its melting point. The separation of the estrone-4-thiourea derivatives (VIII–XI), on the other hand, necessitated the evaporation of the mixture to dryness, without application of heat, and then purifying the residues by precipitation from different cold solvent systems (Table I). *N*-Benzyl-*N'*-(3-hydroxy-17-oxoestra-1,3,5(10)-trien-4-yl)thiourea (XI) was found to be insensitive to heat; hence, its preparation and purification were carried out in boiling ethanol. The structures of V–XI were confirmed by elemental analysis, IR, $^1$H-NMR spectra (Table I), and (for VII and X) MS.

**Biological Screening**—The relative binding affinity of the steroidal thioureas (V–XI) was evaluated by measuring their ability to inhibit the binding of tritiated estradiol to the cytoplasmic estrogen receptor, obtained from immature rat uteri (2, 10). The steroidal thiourea derivatives (V–VII) did not produce any inhibition of binding, indicating that they were totally devoid of binding affinity. Compounds VIII–XI produced only a slight inhibition, indicative of a weak (most probably insignificant) binding affinity (≤0.1%). Moreover, VII and X were evaluated *in vitro* for their inhibition of the growth of cells from the estrogen receptor-positive breast cancer (MCF-7) cell line (12). As shown in Table II, at a concentration of $10^{-6}$ M, both compounds produced a slight inhibition of the growth of MCF-7 cells. These effects were only significant ($p < 0.05$) in the case of VII. In addition, the combination of varying molar concentrations of estradiol with the two thioureas (at a concentration of $10^{-6}$ M) did not suppress the growth inhibition of MCF-7 cells (Table II), indicating that the estrogen receptor was not involved in this weak antitumor activity exhibited by VII and X (12). This behavior totally contrasted with the inhibited growth of MCF-7 cells produced by 1-[2-[*p*-(3,4-dihydro-6-methoxy-2-phenylnaphth-1-yl)phenoxy]ethyl]pyrrolydine hydrochloride (nafoxidine)[1], which was suppressed by addition of estradiol (*cf.*, footnote *b* in Table II). Such a slight inhibition of the growth of MCF-7 cells by the thioureas is consistent with their lack of binding affinity for the estrogen receptor. The presence of the thiourea function, although fulfilling the polar structural requirements for association with the receptor (4, 8, 10), seems to be bulky spatial arrangement as compared with several nitrogen mustard functions (23). Hence, the product was sterically hindered from fitting in the estrogen receptor sites (6).

[1] Lot 100A; The Upjohn Co.

**Table I—Synthesized Estrone-2- and -4-thiourea Derivatives (V–XI)**

| Compound | Yield, % | mp, °C | Molecular[a] Formula | $^1$H-NMR (δ), ppm[b] |
|---|---|---|---|---|
| V | 81 | 186–188[c] | $C_{25}H_{28}N_2O_2S$ | 0.87 (s, 3, C(18)—$CH_3$), 6.62 (s, 1, C(4)-H), 7.30 (br m 5, ArH), 7.71 (s, 1, C(1)—H), 8.81 (s, 2, NHCSNH, disappearing on deuteration), 9.47 (s, 1, OH, lost on deuteration). |
| VI | 96 | 174–176[c] | $C_{26}H_{30}N_2O_2S$ | 0.86 (s, 3, C(18)—$CH_3$), 2.3 (s, 3, tolyl-$CH_3$), 6.75 (s, 1, C(4)—H), 7.1 (m, 4, ArH), 7.49 (s, 1, C(1)—H), 8.38 (s, 1, tolyl-NH, lost on deuteration), 8.51 (s, 1, NH, lost on deuteration), 8.86 (s, 1, OH). |
| VII | 86 | 187–189[c] | $C_{25}H_{27}BrN_2O_2S$ | 0.86 (s, 3, C(18)—$CH_3$), 6.62 (s, 1, C(4)—H), 7.35 (d, 2, $J$ = 10 Hz, ArH), 7.52 (d, 2, $J$ = 10 Hz, ArH), 7.72 (s, 1, C(1)—H), 8.94 (s, 2, NHCSNH, lost on deuteration), 9.6 (s, 1, OH). |
| VIII | 89 | 140–142[d] | $C_{25}H_{28}N_2O_2S$ | |
| IX | 95 | 163–165[d] | $C_{26}H_{30}N_2O_2S$ | 0.88 (s, 3, C(18)—$CH_3$), 2.36 (s, 3, (tolyl-$CH_3$), 6.89 (d, 1, $J$ = 8 Hz, C(2)—H), 7.10–7.41 (m, 6, ArH + C(1)—H + tolyl-NH), 8.02 (br s, 1, NH, lost on deuteration). |
| X | 89 | 161–163[e] | $C_{25}H_{27}BrN_2O_2S$ | |
| XI | 87 | 165–167[f] | $C_{26}H_{30}N_2O_2S$[g] | 0.87 (s, 3, C(18)—$CH_3$), 4.79 (d, 2, $J$ = 6 Hz, $C_6H_5CH_2$, becoming singlet at 4.77 on deuteration), 6.20 (m, 1, benzyl-NH, lost on deuteration), 6.83 (d, 1, $J$ = 8 Hz, C(2)—H), 7.18 (d, 1, $J$ = 8 Hz, C(1)-H), 7.25 (s, 5, ArH), 7.78 (s, 1, NH, lost on deuteration). |

[a] Compounds V–XI were analyzed for C, H, and N; unless otherwise noted, all values were within ±0.4% of the theoretical value. [b] In $CDCl_3$ or $Me_2SO$-$d_6$ plus $D_2O$. [c] The compounds solidified and remelted at 310°C. [d] Recrystallized from benzene–petroleum ether (bp 60–80°C). [e] Recrystallized from chloroform–petroleum ether (bp 60–80°C). [f] Recrystallized from ethanol–water. [g] Calc.: C, 71.86; N, 6.45. Found: C, 71.40; N, 6.00.

## EXPERIMENTAL SECTION[2]

***N*-Substituted *N'*-(3-Hydroxy-17-oxoestra-1,3,5(10)-trien-2-yl)thioureas (V–VII)**—A solution of 2-aminoestrone (II) (200 mg, 0.7 mmol) was dissolved in hot absolute ethanol (15 mL), allowed to cool to room temperature, and then treated with the solution of one molar equivalent of the appropriate arylisothiocyanate derivative in absolute ethanol (5 mL). The solution was allowed to stand at room temperature overnight. The resulting crystals were removed by filtration and washed with petroleum ether. The yields, physical constants, and $^1$H-NMR spectral data of the products are presented in Table I.

**2'-Thio-17-oxoestra-1(10),4-dieno[2,3-d]oxazoline (IV)**—A solution of equimolar amounts of 2-aminoestrone (II) and *m*-tolylisothiocyanate (0.87

[2] All melting points are uncorrected. IR spectra were measured as Nujol mulls on a Beckman 4210 Spectrophotometer. $^1$H-NMR and MS were measured on a Perkin-Elmer R32 and an AEI-MS-50, respectively.

**Table II—Growth Inhibition of MCF-7 Cells by VII and X Alone or Combined with Estradiol**

| Compound | Molarity | DNA, μg[a] | | | |
|---|---|---|---|---|---|
| | | Alone | Estradiol $10^{-8}$ M | Estradiol $10^{-7}$ M | Estradiol $10^{-6}$ M[b] |
| Control | | 9.1 ± 1.8 (100) | | | |
| VII[c] | $10^{-8}$ | 9.6 ± 1.3 (105) | | | |
| | $10^{-7}$ | 9.6 ± 1.2 (105) | | | |
| | $10^{-6}$ | 6.9 ± 0.7 (76) | 6.6 ± 0.9 (72) | 6.0 ± 1.1 (66) | 5.7 ± 0.3 (63) |
| X | $10^{-8}$ | 9.0 ± 1.0 (99) | | | |
| | $10^{-7}$ | 7.8 ± 0.7 (86) | | | |
| | $10^{-6}$ | 7.6 ± 1.4 (83) | 9.6 ± 1.0 (105) | 7.7 ± 1.0 (84) | 7.4 ± 0.5 (81) |

[a] Experiments were performed in quadruplicate; the results are given as mean ± *SD*. The percent of control is given in parentheses. [b] Inhibition produced by $5 \times 10^{-7}$ M nafoxidine alone was 6.2 ± 0.6 (68); the value after addition of $10^{-8}$ M estradiol was 7.6 ± 0.8 (83). [c] Variance analysis showed that VII produced a significant growth inhibition at $10^{-6}$ M ($p < 0.05$); this effect was not suppressed by estradiol. The slight inhibition of X was not significant ($p > 0.10$).

mmol) in ethanol (15 mL) was heated at reflux for 30 min. The solution was concentrated and the product, obtained after cooling, was recrystallized from ethanol to give IV as white shiny scales (95% yield), mp 308–310°C. Compound IV was also produced when thiourea VI was heated in ethanol for recrystallization, or heated above its melting point for a short time. The mixed melting point with a sample of IV, prepared from 2-aminoestrone (II), carbon disulfide, and potassium hydroxide [as previously reported (21)] showed no depression. IR and $^1$H-NMR spectra of IV obtained from both sources were superimposable. IR (mineral oil): $\nu$ 1730 (C=O), 1595 (C=C, Ar), 1510, 1495, 1140, and 930 cm$^{-1}$ (NCS amide I, II, III, and IV bands, respectively); $^1$H-NMR ($CDCl_3$ + $D_2O$): $\delta$ 0.92 (s, 3, C(18)—$CH_3$), 7.05–7.30 (two m, 2, ArH), and 10.55 ppm [br s (diffuse), oxazoline-H, exchangeable].

***N*-Substituted *N'*-(3-Hydroxy-17-oxoestra-1,3,5(10)-trien-4-yl) thioureas (VIII–XI)**—A solution of 4-aminoestrone (III) (200 mg, 0.7 mmol) and the equivalent amount of the isothiocyanate derivative in a mixture of absolute ethanol (15 mL) and acetone (5 mL) was allowed to stand at room temperature for 24–30 h. The solvent was evaporated to dryness without application of heat. The oily residues were dissolved in cold benzene or chloroform (10 mL); the precipitates were removed by filtration and treated with petroleum ether to give the thiourea derivatives (VIII–X) as white amorphous solids. The benzyl derivative XI was prepared by heating at reflux a mixture of 4-aminoestrone (III) (250 mg, 0.87 mmol) and benzylisothiocyanate (130 mg, 0.87 mmol) in absolute ethanol (30 mL) for 30 min. The solution was concentrated to 10 mL, water was added in a dropwise manner, and the solution was allowed to stand overnight to deposit colorless needles. The application of this method to prepare thioureas VIII–X gave impure products. The yields, physical constants, and $^1$H-NMR spectral analyses are recorded in Table I. IR data for the thiourea derivatives (V–XI) (mineral oil): $\nu$ 3340–3310, 3310–3120 (NH), 3120–3050 (OH), 1730–1700 (C=O), 1595–1580 and 1490–1485 (C=C, Ar), 1540–1515, 1340–1330, 1165–1140, and 950–920 cm$^{-1}$ (NCS amide mixed vibrational I, II, III, and IV bands, respectively); MS for VII: *m/z* (relative abundance %); $M^+$ absent, 216 (100), 214 (100), 174 (24), 173 (48), 172 (29), 171 (48), 134 (26), 93 (56), 87 (19), 86 (19), 77 (18), 65 (76); MS for X: 216 (100), 214 (100), 173 (21), 171 (21), 157 (19), 155 (19), 135 (27), 84 (60), 76 (15), 75 (21).

**Relative Binding Affinity for the Estrogen Receptor**—The ability of the compounds to bind to the uterine estrogen receptors was estimated according to a previously described experimental procedure (24). Briefly, uterine estrogen receptor cytosol was incubated at 18°C for 30 min with $5 \times 10^{-9}$ M [$^3$H]estradiol (saturation amount) in the absence or presence of increasing amounts of the test compound (X) or unlabelled estrone (control). After incubation, unbound compounds were removed by dextran-coated charcoal and bound [$^3$H]estradiol was measured. The relative concentration of unlabelled estrone and test compound required to achieve 50% inhibition of the estradiol binding gave the relative binding affinity ($RBA_X = ([I_{50}]\text{estrone}/[I_{50}]_X) \times 100$).

**Effect of VII and X on the Growth of MCF-7 Breast Cancer Cells**—The action of the compounds on MCF-7 cell growth was estimated by measuring the amounts of DNA after 120 h of culture in the absence or presence of the test compound. In practice, MCF-7 cells were grown at 37°C in closed flasks (75 cm$^2$) containing Earle's minimal essential medium supplemented with L-glutamine (0.6 mg/mL), gentamicin (40 μg/mL), penicillin (100 μg/mL), streptomycin (100 μg/mL) and 10% fetal calf serum. At confluency, the cells were removed by trypsinization (0.05% trypsin, 0.025% EDTA) and suspended ($\sim 1 \times 10^5$ cells/mL) in the growth medium supplemented with charcoal-stripped fetal calf serum (0.5% charcoal and 0.005% dextran in 1.5 mL of medium, incubated overnight at 4°C). They were then plated in 35-mm Petri dishes containing this medium and cultured at 37°C in a 95% air–5% $CO_2$ atmosphere. After 24 h, the compounds (solvent: ethanol at the final concentration of 0.1%) were added to the culture dishes. After 48 h, the medium was replaced by fresh medium containing the drugs. The cultures were then grown for an additional 72-h period before final harvest. Both at 24 h (compound addition) and 144 h (end of experiment) the cells were washed twice with 2 mL of Earle's base before being suspended in 1.5 mL of trypsin–EDTA. Total DNA of the collected cells was precipitated in 0.5 M perchloric acid and evaluated by the diphenylamine method (25). The control was run in parallel, without the drug (solvent only added).

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