Synthesis, Uterotrophic, and Antiuterotrophic Activities of Some Estradiol Derivatives Containing Thiadiazole, Thiazoline, and Thiazolidinone Moieties

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Key Words: *Estradiol; thiadiazolylvinylestradiol; thiocarbamoylhydrazonoethylestradiol; thiazolin-2-ylidinehydrazonoethylestradiol; 4-oxothiazolidin-2-ylidinehydrazonoethylestradiol; uterotrophic activity; antiuterotrophic activity*

Summary

The effect of structural modification on the biological activity of hormones has been studied on five novel series of estradiol analogs bearing a variety of substituents at the 2-position of the steroidal nucleus. The synthesized compounds include 2-[2-(5-substituted amino-1,3,4-thiadiazol-2-yl)vinyl]estradiol 17β-acetate **5–9**, 2-aroylmethylestradiols **10–12**, 2-[2-aryl-2-(substituted thiocarbamoylhydrazono)ethyl]estradiols **13–18** and their cyclic thiazoline **19–24**, and thiazolidinone derivatives **25–30**. Among the products, the *p*-hydroxybenzoylmethylestradiol **12** exhibited the highest antiestrogenic activity of 63%. It also elicited 34% of the uterotrophic activity of estradiol.

Introduction

Much concern has been devoted to the synthesis of antiestrogens which constitute an important class of drugs for the endocrine therapy of breast cancer[1–4]. Antiestrogens compete with endogenous estrogens by binding to estrogen receptor (ER) but fail to effectively activate any of the normal transcriptional hormone responses and consequent manifestations of estrogen action[1,4–6].

A structure characteristic of pure steroidal antiestrogens is a bulky side chain with basic or polar functional group that may interact with charged and polar amino acids near the hormone-binding site in the ER[7]. Representative compounds are ICI 164 384[8], EM-139[9], RU 51625[10], or ICI 182 780[4] containing a long alkanamide or alkanesulfoxide side chain at the 7α- or 11β-position of estradiol. These compounds proved to be potent inhibitors of estrogen action and have demonstrated excellent growth-inhibitory effects in both cell and animal models of human breast cancer[2,4,5].

It has long been believed that small alterations in the structure of certain estrogens can greatly affect their receptor binding affinity and biological activity[11,12]. Our recent studies have demonstrated the introduction of various functions in the 2-position of estradiol and estrone. These functions varied between different heterocycles, as pyrazole, isoxazole[13], thiazole[14] or pyrimidine derivatives[15], semicarbazones, thiosemicarbazones, and Mannich bases[16]. Many of the synthesized compounds were found to possess good receptor binding affinity[13], estrogenic[14], and/or antifertility activity[16]. Moreover, blocking the 2-position of estradiol with 1,4-diketone side chain was found to impart a modest antiestrogenic activity in the rat uterine weight test while conversion of such polar and flexible side chain into rigid 2-pyridazinyl ring system completely abolished estrogenic and antiestrogenic activity[17].

The present study describes the synthesis of five novel series of estradiol analogs bearing various basic and polar functional groups in the 2-position while keeping positions 3 and 17 of estradiol free for binding to receptor sites. In one series, the functionalities include a carboxylic group or various thiadiazoles attached to the steroidal nucleus by a vinyl chain. In the others, the chain carries various phenacyl, thiosemicarbazone moieties, thiazoline, and thiazolidinone derivatives. These substituents with flexible straight chains or rigid heterocyclic systems will attain different spatial arrangements and, in turn, variously affect the binding affinity of the products to the receptors and the estrogenic or antiestrogenic property.

Chemistry

2-Formylestradiol **2** was prepared by a modified Sommelet reaction[18] involving the treatment of 2-dimethylaminomethylestradiol **1**[18–20] with hexamethylenetetramine in acetic acid. Acetylation of **2**[13] followed by condensation of the formed 2-formylestradiol-3,17β-diacetate **3** with acetic anhydride in the presence of anhydrous sodium acetate, under Perkin reaction conditions, gave 3-(3,17β-diacetyloxyestra-1,3,5(10)trien-2-yl)prop-2-enoic acid **4** (Scheme 1). The ^{1}H-NMR spectrum of **4** lacked the singlet at 9.81 ppm for the aldehydic proton of **2** and showed a singlet at 12.28 ppm for the carboxylic proton together with two doublets at 6.85 and 8.01 ppm (J = 16 Hz) for the vinylic protons in *E* configuration.

Heating **4** with thiosemicarbazide or the appropriate N^4-substituted thiosemicarbazide in the presence of phosphorus oxychloride[21] gave the steroidal vinylthiadiazole derivatives **5–9**. After purification by preparative TLC, compounds **5–9** were identified as the 3-hydroxy-17β-acetate derivatives, as evidenced by IR and ^{1}H-NMR. The 3-acetate function underwent hydrolysis during the separation process.

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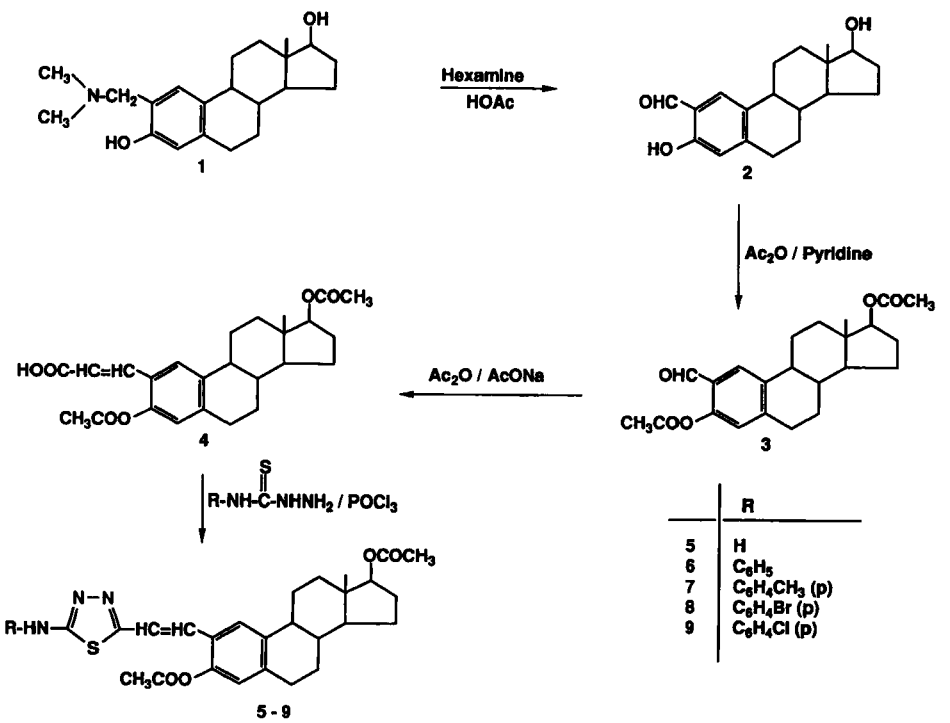
Table 1. Preparative and analytical data of the synthesized compounds **5–30**.

Comp. No.	Molecular formula (M.Wt.)	Yield [%]	R_f	^{1}H-NMR[a] (δ ppm) and/or MS (70 eV) *m/z* (relative abundance)
5	$C_{24}H_{29}N_3O_3S$ (439)	33	0.45	**MS:** 439(23)[M^+], 396(100)[M^+-$COCH_3$].
6	$C_{30}H_{33}N_3O_3S$ (515)	29	0.43	**MS:** 439(52) [M^+-C_6H_5, +H], 395(39) [M^+-C_6H_5,-$COCH_3$].
7	$C_{31}H_{35}N_3O_3S$ (529)	32	0.36	**^{1}H-NMR:** 2.03 (s,3H,17β-$OCOCH_3$), 2.97 (s,3H,*p*-tolyl CH_3), 6.29 (d,1H, *J* = 16 Hz, vinyl-*CH*=CH), 7.15 (d,2H, *J* = 8 Hz, Ar-H ortho to CH_3), 7.43 (d,2H, *J* = 8 Hz, Ar-H meta to CH_3), 7.55 (s,1H,C-1-H), 7.81 (d,1H, *J* = 16 Hz, vinyl-CH=*CH*), 8.65 (s,1H,NH).
8	$C_{30}H_{32}BrN_3O_3S$ (594)	39	0.39	**^{1}H-NMR:** 2.00 (s,3H,17β-$OCOCH_3$), 6.23 (d,1H, *J* = 16 Hz, vinyl-*CH*=CH), 6.93 (d,2H, *J* = 9 Hz, Ar-H meta to Br), 7.18 (d,2H, *J* = 9 Hz, Ar-H ortho to Br), 7.38 (s,1H,C-1-H), 7.78 (d,1H, *J* = 16 Hz, vinyl-CH=*CH*), 8.34 (s,1H,NH), 12.14 (s,1H,3-OH). **MS:** 595(100) [M^++1], 593(98)[M^+], 439 (21) [M^+-C_6H_4Br(p), +H], 396(66) [M^+-C_6H_4Br(p), -$COCH_3$,+H].
9	$C_{30}H_{32}ClN_3O_3S$ (549.5)	37	0.41	**^{1}H-NMR:** 2.31 (s,3H,17β-$OCOCH_3$), 6.46 (d,1H, *J* = 16 Hz, vinyl-*CH*=CH), 7.33 (d,2H, *J* = 8 Hz, Ar-H meta to Cl), 7.61 (d,2H, *J* = 8 Hz, Ar-H ortho to Cl), 7.72 (s,1H,C-1-H), 7.86 (d,1H, *J* = 16 Hz, vinyl-CH=*CH*), 10.21 (s,1H,NH), 11.85 (s,1H,3-OH). **MS:** 551(1.2) [M^++2], 549(4)[M^+], 438 (49) [M^+-C_6H_4Cl(p)], 395(100) [M^+-C_6H_4Cl (p),-$COCH_3$].
10	$C_{26}H_{30}O_3$ (390)	64	0.25	**^{1}H-NMR**: 3.61 (s,2H,CH_2), 6.93–7.39 (m,5H,Ar-H), 7.45 (s,1H,C-1-H). **MS:** 391(4)[M^++1], 312 (100)[M^+-C_6H_5,-H].
11	$C_{26}H_{29}ClO_3$ (424.5)	68	0.28	**MS:** 312(100)[M^+-C_6H_4Cl (p),-H].
12	$C_{26}H_{30}O_4$ (406)	58	0.32	**^{1}H-NMR:** 3.84 (s,2H,CH_2), 7.28 (s,1H,C-1-H), 7.45–8.11 (m,4H,Ar-H), 10.9 (s,1H, *p*-OH). **MS:** 407(14)[M^++1], 313(100) [M^+-C_6H_4 OH(p)].
13	$C_{33}H_{37}N_3O_2S$ (539)	31	0.34	**^{1}H-NMR:** 2.16 (s,2H,CH_2), 6.99–7.52 (m,10H,Ar-H), 7.67 (s,1H,C-1-H), 9.87 (s,1H,N^4-H exchangeable with D_2O), 11.75 (s,1H,N^2-H exchangeable with D_2O). **MS:** 538 (7.5)[M^+-1], 413 (12.3) [M^+-$C_6H_5NH_2$,-SH], 336(100) [M^+-$C_6H_5NH_2$,-SH,-C_6H_5].
14	$C_{33}H_{36}ClN_3O_2S$ (573.5)	27	0.32	**MS:** 414 (5.1)[M^+- *p*-$ClC_6H_4NH_2$, -SH,+H], 336 (100)[M^+-*p*-Cl- $C_6H_4NH_2$,-SH,-C_6H_5].
15	$C_{33}H_{36}ClN_3O_2S$ (573.5)	35	0.4	**MS:** 575 (0.6)[M^++2] 573 (1.9)[M^+], 482 (1.3) and 480 (3.8)[M^+-$C_6H_5NH_2$], 336 (100)[M^+-$C_6H_5NH_2$,-SH,-C_6H_4Cl(p)].
16	$C_{33}H_{35}Cl_2N_3O_2S$ (608)	36	0.19	**^{1}H-NMR:** 2.31(s,2H,CH_2), 6.91–8.19 (m,9H,8Ar-H overlaping with C-1-H), 10.15 (s,1H,N^4-H exchangeable with D_2O), 11.23 (s,1H,N^2-H exchangeable with D_2O). **MS:** 482 (4.2) and 480 (12.3)[M^+- *p*-$ClC_6H_4NH_2$], 338 (30)[M^+- *p*-Cl C_6H_4 NH_2, -SH,-C_6H_4Cl(p),+2H].
17	$C_{33}H_{37}N_3O_3S$ (555)	33	0.15	**MS:** 431 (9.5) [M^+- $C_6H_5NH_2$, -SH,+2H], 335 (5.0)[M^+-$C_6H_5NH_2$, -SH, -C_6H_4OH(p),-H].
18	$C_{33}H_{36}ClN_3O_3S$ (589.5)	38	0.26	**^{1}H-NMR:** 2.28 (s,2H,CH_2), 6.81–8.08 (m,9H,8Ar-H overlaping with C-1-H), 10.01 (s,1H,N^4-H exchangeable with D_2O), 11.8 (s,1H,N^2-H exchangeable with D_2O), 11.95(s,1H,*p*-OH exchangeable with D_2O). **MS:** 591 (4.3)[M^++2], 589 (12.4) [M^+], 336 (78.5) [M^+- *p*-$ClC_6H_4NH_2$,-SH, -C_6H_4 OH(p)].
19	$C_{41}H_{41}N_3O_2S$ (639)	33	0.58	**^{1}H-NMR:** 2.28 (s,2H,CH_2), 5.80 (s,1H, thiazoline C-5-H), 6.82–7.71 (m,16H,15 Ar-H overlapping with C-1-H). **MS:** 639 (19.7)[M^+], 556 (28.3) [M^+- C_6H_5,-6H], 367 (20.2) [M^+-$C_6H_5NH_2$, -2x C_6H_5, -C_2H_4, + 3H].
20	$C_{41}H_{40}ClN_3O_2S$ (673.5)	39	0.52	**MS:** 563 (2.1)[M^+-C_6H_4Cl(p),-H], 477 (8.5) [M^+-C_6H_4Cl(p), -C_6H_5, - 8H], 439 (100) [M^+ - *p*-$ClC_6H_4NH_2$, -C_6H_5,-C_2H_4, - 2H].
21	$C_{41}H_{40}ClN_3O_2S$ (673.5)	25	0.61	**MS:** 675 (8.5)[M^++2], 673 (26.4)[M^+], 364 (100) [M^+-$C_6H_5NH_2$, -C_6H_5, .C_2H_4, -C_6H_4Cl(p)].
22	$C_{41}H_{39}Cl_2N_3O_2S$ (708)	26	0.6	**^{1}H-NMR:** 2.31 (s,2H,CH_2), 5.79 (s,1H, thiazoline C-5-H), 6.97–8.21 (m,14H,13 Ar-H overlaping with C-1-H). **MS:** 599 (1.1) and 597 (3.6) [M^+- -C_6H_4Cl(p),+H],541 (17.0) M^+- C_6H_4Cl(p), -Cl,- 18-CH_3, -5H], 440 (29.8) [M^+-*p*-$ClC_6H_4NH_2$, - C_6H_5, .C_2H_4, -Cl].

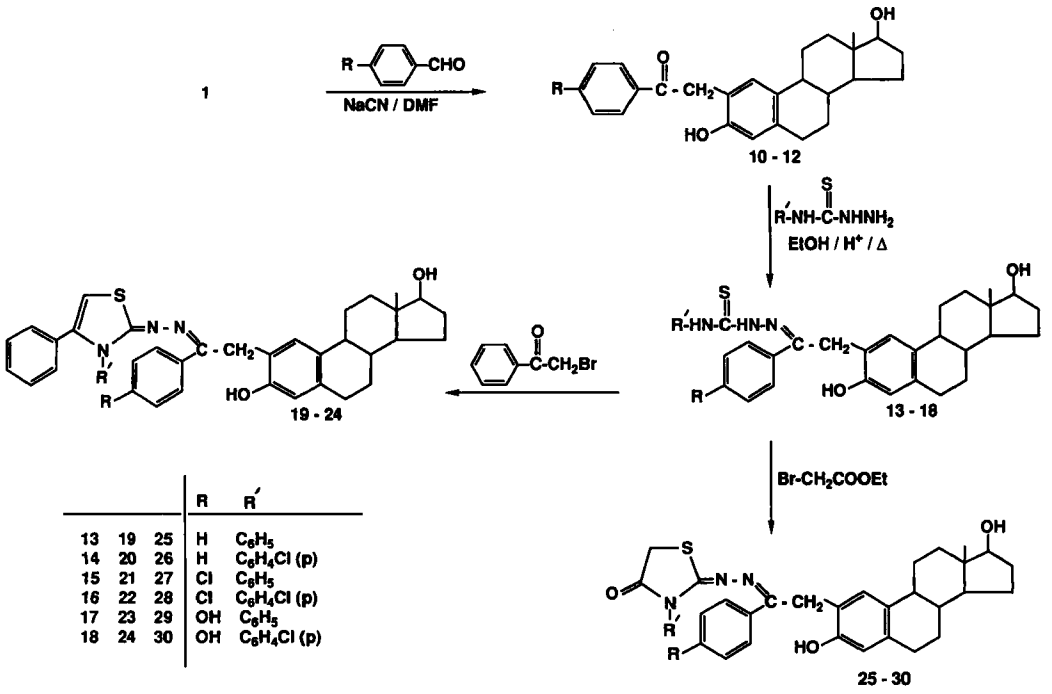
Table 1. *Continued.*

Comp. No.	Molecular formula (M.Wt.)	Yield [%]	R_f	^{1}H-NMR[a] (δ ppm) and/or MS (70 eV) *m/z* (relative abundance)
23	$C_{41}H_{41}N_3O_3S$ (655)	43	0.56	**^{1}H-NMR:** 2.71 (s,2H,CH_2), 5.82 (s,1H, thiazoline C-5-H), 6.81–8.42 (m,15H,14 Ar-H overlapping with C-1-H), 10.85 (s,1H,*p*-OH). **MS:** 657 (2.2)[M^++2], 570 (3.3) [M^+- C_6H_5,-8H], 466 (8.3) [M^+- $C_6H_5NH_2$, - C_6H_5, -C_2H_4, +9H].
24	$C_{41}H_{40}ClN_3O_3S$ (689.5)	35	0.54	**MS:** 570 (4.8)[M^+-C_6H_4Cl(p),-8H], 457 (2.1) [M^+-*p*-Cl $C_6H_4NH_2$,-C_6H_5,- C_2H_4].
25	$C_{35}H_{37}N_3O_3S$ (579)	28	0.73	**MS:** 578 (71)[M^+-1], 440 (34.1)[M^+- C_6H_5NCO,-CH_3,-5H], 355 (100) [M^+- C_6H_5 NCO,-CH_3,- C_6H_5,- 18-CH_3,+2H].
26	$C_{35}H_{36}ClN_3O_3S$ (613.5)	35	0.77	**MS:** 615 (3.1)[M^++2], 613 (10.2) [M^+] , 501 (1.3) [M^+- C_6H_4 Cl(p),-H].
27	$C_{35}H_{36}ClN_3O_3S$ (613.5)	31	0.71	**MS:** 615 (1.8)[M^++2], 613 (5.4) [M^+], 539 (0.4) and 537 (1.5)[M^+-C_6H_5,+H], 440 (6.5) [M^+-C_6H_5NCO,-CH_3,-Cl,-4H], 354 (89.9) [M^+-C_6H_5NCO,-CH_3, -C_6H_4Cl(p), -18-CH_3,+H].
28	$C_{35}H_{35}Cl_2N_3O_3S$ (648)	41	0.69	**^{1}H-NMR:** 2.22 (s,2H,CH_2), 3.92 (s,2H, thiazolidinone C-5-H), 7.08 (s,1H,C-1-H), 7.28–7.91 (m,8H,Ar-H). **MS:** 539 (2.2) and 537 (6.9)[M^+-C_6H_4Cl (p),+H], 422 (78.1) [M^+-2xC_6H_4Cl(p),-3H], 350 (23.6) [M^+- *p*-ClC_6H_4NCO,-CH_3,-C_6H_4Cl (p),-18-CH_3,-3H].
29	$C_{35}H_{37}N_3O_4S$ (595)	35	0.75	**MS:** 509 (3.0)[M^+-C_6H_5, -9H], 355 (100) [M^+-C_6H_5NCO,-CH_3,-C_6H_4OH(p), -18-CH_3,+2H].
30	$C_{35}H_{36}ClN_3O_4S$ (629.5)	38	0.67	**^{1}H-NMR:** 2.17 (s,2H,CH_2), 4.02 (s,2H, thiazolidinone C-5-H), 7.25–8.17 (m,9H, 8Ar-H overlaping with C-1-H). **MS:** 518 (14.3) [M^+-C_6H_4Cl (p)], 456 (100) [M^+- *p*-ClC_6H_4NCO, -CH_3,-5H], 348 (9.4)[M^+- *p*-ClC_6H_4NCO, -CH_3, -C_6H_4OH (p), - 18CH_3,-5H].

[a] Common chemical shifts (δ ppm) shown by all synthesized compounds **5–30**. 0.81–0.89 (s,3H,18-CH_3), 4.28–4.48 (t,dist,1H, J = 6 Hz, 17α-H), 6.54–6.89 (s,1H,C-4-H).



Scheme 1



			R	R′
13	19	25	H	C_6H_5
14	20	26	H	C_6H_4Cl (p)
15	21	27	Cl	C_6H_5
16	22	28	Cl	C_6H_4Cl (p)
17	23	29	OH	C_6H_5
18	24	30	OH	C_6H_4Cl (p)

Scheme 2

On the other hand, treatment of 2-dimethylaminomethylestradiol **1** with a mixture of benzaldehyde or *p*-substituted benzaldehyde and sodium cyanide in DMF[22], gave the 2-aroylmethylestradiol derivatives **10–12**. The IR spectra showed the carbonyl absorption between 1696–1682 cm^{-1}, while ^{1}H-NMR showed the methylene and substituted aryl protons resonating at their expected chemical shifts. Condensation of compounds **10–12** with phenyl or *p*-chlorophenylthiosemicarbazide in boiling absolute EtOH containing few drops of glacial acetic acid gave the thiosemicarbazone derivatives **13–18** (Scheme 2). The ^{1}H-NMR spectra for compounds **13,16** and **18**, as representative examples, showed the NH exchangeable protons as two downfield singlets. The N^4-H resonated between 9.87–10.15 ppm while N^2-H, being more deshilded, appeared between 11.23–11.80 ppm. Cyclization of the thiosemicarbazones **13–18** with phenacyl bromide or ethyl bromoacetate in the presence of anhydrous sodium acetate gave the thiazoline **19–24** and thiazolidinone derivatives **25–30**, respectively. ^{1}H-NMR spectra of the products lacked the NH protons present in the starting material and showed the singlet for the thiazoline C-5-proton at 5.79–5.82 ppm and that for the thiazolidinone C-5-protons at 3.92–4.02 ppm.

Results and Discussion

To assess whether substitution in the 2-position of the estradiol skeleton is inhibitory for estrogenic activity, as has been previously thought[11], we have prepared several estradiol derivatives having in the 2-position various straight chains or heterocyclic systems. The chains include the steroidal propenoic acid **4** and the thiosemicarbazone derivatives **13–18**, while the heterocyclic systems include the steroidal thiadiazoles **5–9**, thiazolines **19–24** and thiazolidinones **25–30**. The products were *in vivo* evaluated for estrogenic and antiestrogenic activity to assess how much structure modification in the 2-position of estradiol would affect its biological activity.

The results of biological screening (Table 2) showed that the 2-formylestradiol-3,17β-diacetate **3**, at a dose of 0.09 μmol/rat/day, produced a 37% increase in uterine weight relative to estradiol and was almost (14%) devoid of antiuterotrophic activity. Converting the formyl group into a longer polar chain, propenoic acid moiety, (compound **4**) caused an increase (57%) in the antiuterotrophic activity of the product but without any agonistic effect. Both the uterotrophic and antiuterotrophic activities decreased for compounds **5–9** in which a rather bulky and rigid thiadiazole ring is placed in the 2-position of estradiol.

On the other hand, introduction of various phenacyl functions in the 2-position of estradiol (compounds **10–12**) induced a weak uterotrophic activity with a relatively high antiuterotrophic property. Compound **12**, having a *p*-hydroxyphenyl side chain, elicited the highest (63%) antiuterotrophic activity. Replacement of the keto function in compounds **10–12** with different thiosemicarbazone moieties (compounds **13–18**) diminished the antiuterotrophic activity but retained uterotrophic property. Compounds **15** and **18** showed a moderate 50% and 54% uterotrophic activity, respectively. In contrast, compound **24**, among the cyclic thiazoline derivatives (compounds **19–24**), elicited 51% antiuterotrophic activity with 33% uterotrophic activity. In the thiazolidinones **25–30**, only compound **26** exhibited 59% uterotrophic activity.

Table 2: Uterotrophic and antiuterotrophic activity of the synthesized compound **3–30** in immature female albino rats.

Comp.	Uterotrophic activity		Antiuterotrophic activity	
	Dry uterine weight (mg/100 g)	%Uterotrophic activity	Dry uterine weight (mg/100 g)	%Antiuterotrophic activity
Control[a]	5.5 ± 0.1	–	7.0 ± 0.3	–
Estradiol	49.2 ± 3.2[d]	100	45.5 ± 2.1[e]	–
3	21.5 ± 0.4[e]	37	40.3 ± 2.2[e]	14
4	15.4 ± 2.8[c]	22	23.6 ± 1.1[e]	57
5	10.1 ± 0.6[e]	10	30.2 ± 3.1[e]	40
6	13.0 ± 0.6[e]	17	30.5 ± 0.4[e]	39
7	10.9 ± 0.5[e]	12	31.5 ± 0.3[e]	37
8	14.9 ± 1.6[e]	21	34.4 ± 2.7[e]	29
9	11.0 ± 2.3	12	29.8 ± 1.9[e]	41
10	22.7 ± 2.4[e]	39	24.8 ± 1.7[e]	54
11	18.4 ± 1.3[e]	29	25.0 ± 1.1[e]	53
12	20.2 ± 0.5[e]	34	21.1 ± 0.2[e]	63
13	18.3 ± 1.2[e]	29	26.9 ± 1.3[e]	48
14	21.1 ± 0.8[e]	36	28.4 ± 0.3[e]	44
15	27.4 ± 2.1[e]	50	29.4 ± 1.6[e]	42
16	23.5 ± 0.8[c]	41	29.0 ± 0.8[e]	43
17	24.3 ± 1.0[e]	43	27.6 ± 2.5[e]	47
18	28.9 ± 2.8[e]	54	31.7 ± 1.7[e]	36
19	22.6 ± 2.1[e]	39	28.4 ± 1.1[e]	44
20	21.4 ± 0.9[e]	36	29.0 ± 0.7[e]	43
21	10.6 ± 0.2[e]	12	27.1 ± 2.3[e]	48
22	22.7 ± 0.7[e]	39	27.0 ± 0.3[e]	48
23	20.8 ± 0.5[e]	35	29.6 ± 0.5[e]	41
24	19.9 ± 1.0[e]	33	25.9 ± 1.1[e]	51
25	24.0 ± 0.4[e]	42	33.9 ± 1.4[e]	30
26	31.2 ± 2.6[e]	59	33.7 ± 2.4[e]	31
27	16.9 ± 2.0[d]	26	28.9 ± 1.9[e]	43
28	29.1 ± 0.3[e]	54	34.6 ± 0.3[e]	28
29	25.5 ± 1.7[e]	46	39.5 ± 1.9[e]	16
30	26.6 ± 0.4[e]	48	29.5 ± 0.4[e]	42

Number of animals in each group = 4

Dose of estradiol = 0.09 µmol/rat/day.

Doses of the tested compounds were calculated on an molar ratio basis.

[a]Rats received vehicle (DMSO) and served as control

All the tested compounds exhibited dry uterine weight/wet uterine weight%between 25–30%.

Results were expressed as mean ± SEM. Data were analysed by one way variance. Student's t test for unpaired observations was used.

Differences between means were conisdered significant if [b] $p < 0.05$. [c] $p < 0.02$. [d] $p < 0.01$. [e] $p < 0.001$.

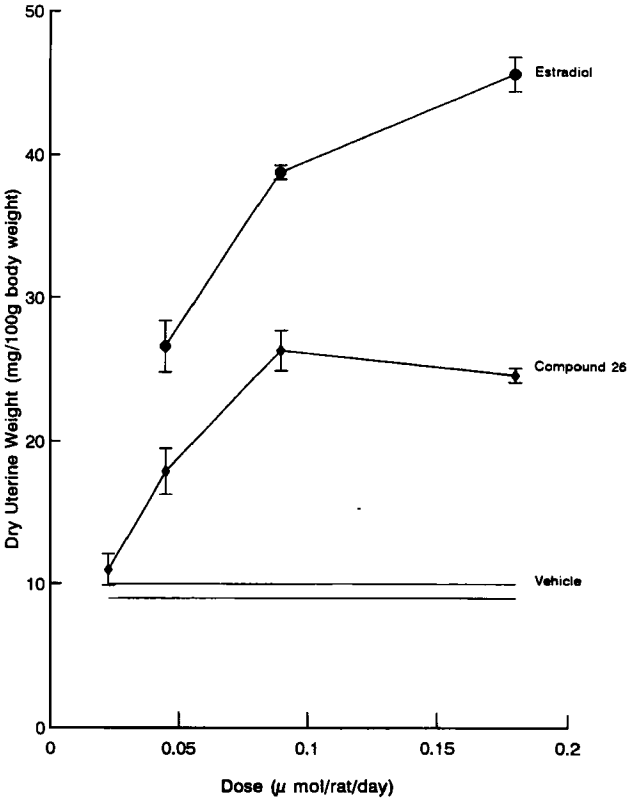


Fig. 1. Uterotrophic activity of estradiol and compound **26** in immature female albino rats. Results are expressed as Mean ± SEM.

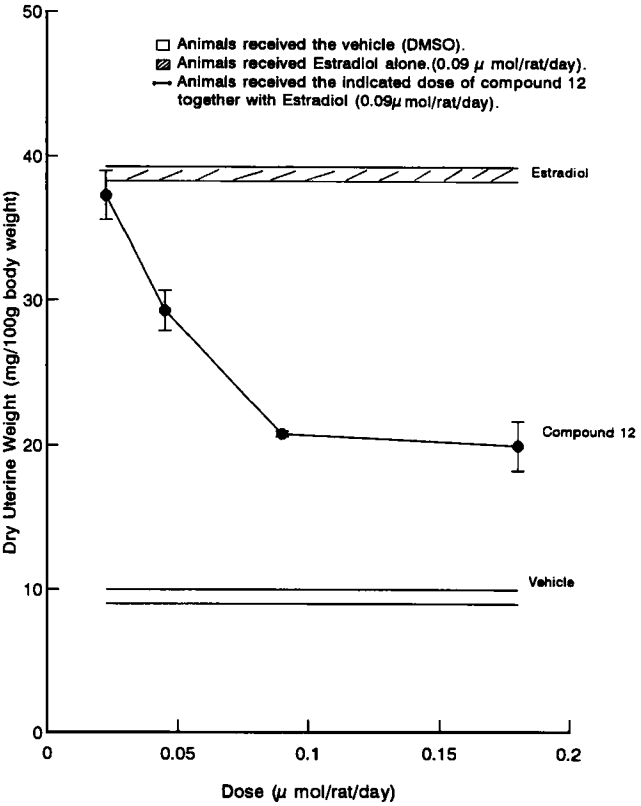


Fig. 2. Dose-response curve of the antiuterotrophic activity of compound **12** in immature female albino rats. Results are expressed as Mean ± SEM.

Summing up the results (Table 2), compounds **26** and **12** are the most active among the series. The former produced the highest uterotrophic (59%) and the latter produced the highest (63%) antiuterotrophic response.

A more precise assessment of the uterotrophic activity of **26** and antiuterotrophic activity of **12** is illustrated in Fig. 1 and 2, respectively. The uterotrophic effect in reponse to different doses (0.0225–0.18 μmol/rat/day) of 2-[2-phenyl-2-(3-*p*-chlorophenyl-4-oxothiazolidin-2-ylidinehydrazono)-ethyl]estradiol **26** was compared to that of estradiol (0.045–0.18 μmol/rat/day). Compound **26** at a dose of 0.0225, 0.045, and 0.09 μmol/rat/day caused a 5%, 29%, and 57% uterotrophic effect, respectively (Fig. 1). At much higher dose (0.18 μmol/rat/day), compound **26** did not produce a greater uterotrophic response.

On the other hand, the antiuterotrophic activity of 2-*p*-hydroxybenzoylmethylestradiol **12** was studied by simultaneous administration of various doses (0.0225–0.18 μmol/rat/day) of this compound and a standard dose of estradiol (0.09 μmol/rat/day). Compound **12** at 0.0225–0.045μmol/rat/day caused a dose-related inhibition of estradiol-induced increase in uterine weight (Fig. 2). A maximal inhibition (61%)was attained at a dose of 0.09 μmol/rat/day. At much higher dose (0.18 μmol/rat/day), compund **12** produced almost the same antiuterotrophic activity (64%), indicating its partial but significant antiestrogenic effect.

The results obtained in this study have indicated that these newly synthesized compounds, unlike pure antiestrogens, did not show complete separation of estrogenic from antiestrogenic activity. They have, however, shed some light on the changes in biological activities due to varying structural and electronic nature of substituents in the 2-position of estradiol.

Acknowledgement

The author thanks the members of the Pharmacology Department, Faculty of Pharmacy, University of Alexandria for their assistance and advice during the biological experiments.

Experimental Part

Melting Points: Griffin melting point apparatus, uncorrected.- IR spectra: Perkin-Elmer FT-IR 1650 (KBr).-^{1}H-NMR spectra ($CDCl_3$) Varian EM-390 spectrometer 90 MHz or Jeol 90 QFT 90 MHz, TMS as internal standard. The chemical shifts are given in ppm δ values (s, singlet; d, doublet; t, triplet; m, multiplet and dist, distorted).- MS: Shimadzu GCMS QP 1000 EX gas chromatograph-mass spectrometer. The homogeneity of products was checked by ascending TLC run on silica gel G (Merck 60) coated glass plates.- Preparative TLC was performed on 20×20 cm^2 plates coated with 30 g silica gel 60 GF 254 for TLC (Adwic Laboratory Chemicals, Egypt).- A duo-UV lamp (Desaga, Heidelberg, Germany) was used for location of the spots.- Microanalysis: Microanalytical Unit, Faculty of Science, Cairo University, Egypt.

2-Formylestradiol **2**

Compound **2** was prepared from 2-dimethylaminomethylestradiol **1** [18–20] according to the previously described method[18]; mp: 239–241 °C (ref [18] 240–242 °C , ref [23] 231–233 °C).- IR: ν = 3422 (OH), 1719 (C=O), 1634 cm^{-1} (C=C aromatic). -^{1}H-NMR: δ = 0.83 (s,3H,18-CH_3), 4.58 (t,dist.,1H, *J* = 6 Hz,17α-H), 7.18 (s,1H,C-4-H), 7.93 (s,1H,C-1-H), 9.79 (s,1H,CHO).

2-Formylestradiol 3,17β-diacetate **3**

A solution of 2-formylestradiol **2** (0.5 g, 1.66 mmol) in a mixture of dry pyridine (5 ml) and acetic anhydride (5 ml) was heated under reflux for 1 h. After cooling to room temp., the mixture was poured onto ice-cold water to separate a yellow oil which was extracted with $CHCl_3$ (2×20 ml), dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by chromatography on a column of silica gel. Elution with a mixture of C_6H_6 : $CHCl_3$: EtOAc (2.5 : 2.5 : 1, *v/v/v*) yielded 0.43 g (67.1%) of the diacetate **3** : $C_{23}H_{28}O_5$ (384) as a viscous oil.- R_f : 0.48.- IR: ν = 1758 (C=O, C-3-acetate), 1728 (C=O, C-17-acetate), 1696 (C=O, 2-formyl), 1600–1531 (C=C aromatic), 1240 and 1200 cm^{-1} (C-O-C).-^{1}H-NMR: δ = 0.85 (s,3H,18-CH_3), 2.09 (s,3H,17β-$OCOCH_3$), 2.36 (s,3H,3-$OCOCH_3$), 4.74 (t,dist.,1H, *J* = 6 Hz, 17α-H), 6.91 (s,1H,C-4-H), 7.85 (s,1H,C-1-H), 9.81 (s,1H,2-formyl).- MS (70 eV); *m/z* (%) = 386 (1.7) [M^++2], 312 (100) [M^+-18-CH_3, -$OCOCH_3$, +2H].

3-(3,17β-diacetyloxyestra-1,3,5(10)trien-2-yl)prop-2-enoic acid **4**

A solution of 2-formylestradiol 3,17β-diacetate **3** (0.7 g, 1.82 mmol) in acetic anhydride (5 ml) was treated with dry, pulverized, freshly fused sodium acetate (0.15 g, 1.82 mmol). The reaction mixture was refluxed for 6 h with stirring. After cooling, the reaction mixture was poured onto ice-water and left for 1h. The separated product was filtered, washed with water, dried and purified by column chromatography using C_6H_6 : EtOAc (9.5 : 0.5, *v/v*) as eluent to give 0.33 g (42.5%) of **4** : $C_{25}H_{30}O_6$ (426) as a viscous oil.- R_f : 0.73.- IR : ν = 3450 (*COOH*), 1762 (C=O, C-3-acetate), 1738 (C=O, C-17-acetate), 1657 (*CO*OH), 1592 and 1531 (C=C aromatic), 1240 and 1198 cm^{-1} (C-O-C).-^{1}H-NMR: δ = 0.83 (s,3H, 18-CH_3), 2.07 (s,3H, 17β-$OCOCH_3$), 2.34 (s,3H,3-$OCOCH_3$), 4.56 (t,dist.,1H, *J* = 6 Hz,17α-H), 6.67 (s,1H,C-4-H), 6.85 (d,1H, *J* = 16 Hz, CH=*CH*COOH), 7.69 (s,1H,C-1-H), 8.01 (d,1H, *J* = 16 Hz, *CH*=CHCOOH), 12.28 (s,1H,COOH).- MS (70 eV); *m/z* (%) = 427 (0.2) [M^++1], 396 (29)[M^+-O_2, + 2H], 354 (100)[M^+-O_2, -$COCH_3$, + 4H].

General procedure for preparation of 2-[2-(5-substituted amino-1,3,4-thiadiazol-2-yl)vinyl]estradiol 17β-acetate **5–9**

Phosphorus oxychloride (5 ml) was gradually added with continous stirring and cooling to a mixture of **4** (0.3 g, 0.7 mmol) and thiosemicarbazide or the appropriate N^4-substituted thiosemicarbazide (0.7 mmol). The reaction mixture was heated under reflux for 1h. Excess phosphorus oxychloride was distilled off under vacuum and the oily residue was poured dropwise while stirring onto ice-cold 4% $NaHCO_3$ solution. The separated product was extracted with $CHCl_3$ (2×20 ml), washed with water, dried (anhydrous Na_2SO_4), evaporated under vacuum and purified by preparative TLC using $CHCl_3$: EtOAc : C_6H_6 (5:1:5,*v/v/v*) as the developing solvent. The products were obtained as yellow viscous oils, except for compound **7** (white crystals; mp: 128–131 °C; -Analysis calcd for $C_{31}H_{35}N_3O_3S$ (529): N, 7.93; S, 6.04. Found: N, 7.9; S, 6.6).- IR : ν = 3480–3472 (3-OH), 3320–3315 (HN), 1734–1730 (C=O, C-17-acetate), 1587–1510 cm^{-1} (C=N and C=C aromatic). The preparative and analytical data of the products are reported in Table 1.

General procedure for preparation of 2-aroylmethylestradiol **10–12**

A solution of benzaldehyde or *p*-substituted benzaldehyde (3 mmol) in dry DMF (5 ml) was added dropwise over a period of 1.5h to a stirred mixture of sodium cyanide (0.074 g, 1.5 mmol) in the same solvent (5 ml). The mixture was heated at 35 °C (external temp.) and stirring was continued at the same temp. for an additional 30 min. A solution of 2-dimethylaminomethylestradiol **1** (0.5 g, 1.52 mmol) in dry DMF (5 ml) was added dropwise to the above mixture within 2h while the temp. was maintained at 100 °C (external temp.). After stirring at the same temp. for further 1h, the reaction mixture was allowed to cool and poured onto 10% HCl (100 ml). The product was extracted with $CHCl_3$ (3×20 ml), washed with water, dried (anhydrous Na_2SO_4), evaporated under vacuum and purified by column chromatography using C_6H_6: EtOAc (8:2, *v/v*) as eluent. The products were obtained as yellow viscous oils.- IR: ν = 3357–3335 (3-OH), 1696–1682 (C=O), 1618–1591, 1533–1503 cm^{-1} (C=C aromatic). The preparative and analytical data of the products **10–12** are reported in Table 1.

General procedure for preparation of 2-[2-aryl-2-(substituted thiocarbamoylhydrazono)ethyl]estradiol **13–18**

A mixture of appropriate 2-aroylmethylestradiol **10–12** (0.3 g) and equimolar amount of phenylthiosemicarbazide or *p*-chlorophenylthiosemicarbazide in absolute EtOH (15 ml) containing three drops of glacial acetic acid was heated under reflux for 8h. The reaction mixture was concentrated to small volume and left to cool at room temp. for an overnight. The separated precipitate was filtered, dried and purified by preparative TLC using C_6H_6 : EtOAc (8:2, *v/v*) as developing system to give the desired thiosemicarbazone derivatives **13–18** as yellow viscous oils.– IR: ν = 3530-3420 (3-OH), 3320-3280 (NH), 1608–1597 (C=N), 1515–1490 (C=C aromatic), 1545–1525,1336–1315,1249–1239,870-848 cm^{-1} (NCS amide I,II,III and IV bands). The preparative and analytical data are reported in Table 1.

General procedure for preparation of 2-[2-Aryl-2-(3-substituted-4-phenylthiazolin-2-ylidinehydrazono)ethyl]estradiol **19–24**

To a solution of the appropriate thiosemicarbazone derivatives **13–18** (0.742 mmol) in absolute EtOH (15 ml), were added phenacyl bromide (0.147 g, 0.742 mmol) and anhydrous sodium acetate (0.06 g, 0.742 mmol). The reaction mixture was heated under reflux for 6h, partially concentrated and left to cool overnight. The formed precipitate was filtered and purified by preparative TLC using C_6H_6: EtOAc (8:2, *v/v*) as developing solvent to afford the products **19–24** as yellow viscous oils.– IR: ν = 3426–3338 (OH), 1655–1640 (C=N), 1595–1588,1510–1492 cm^{-1} (C=C aromatic). The preparative and analytical data are reported in Table 1.

General procedure for preparation of 2-[2-aryl-2-(3-substituted-4-oxothiazolidin-2-ylidinehydrazono)ethyl]estradiol **25–30**

A solution of the appropriate thiosemicarbazone derivatives **13–18** (0.742 mmol) in absolute EtOH (15 ml) was treated with ethyl bromoacetate (0.124 g, 0.742 mmol) and anhydrous sodium acetate (0.06 g, 0.742 mmol). The reaction mixture was heated under reflux for 8h, the solvent was evaporated under reduced pressure and the residue was extracted with $CHCl_3$ (3×15 ml). The chloroformic layer was washed with water, dried (anhydrous Na_2SO_4) and evaporated under vacuum. The products **25–30** were purified by preparative TLC using C_6H_6 : EtOAc : $CHCl_3$ (5:1:5, *v/v/v*) as developing solvent and were obtained as yellow visous oils.– IR : ν = 3410–3345 (OH), 1734–1725 (thiazolidinone C=O), 1648–1635 (C=N), 1578–1565,1503–1478 cm^{-1} (C=C aromatic). The preparative and analytical data of the products are reported in Table 1.

Biological Activity

Uterotrophic Activity

The uterotrophic activity[6,16,24] of the synthesized compounds was evaluated by determining the uterine weight gain in immature albino female rats (approximately 45–60 g, 21–23 days of age), obtained from the animal house of the Faculty of Pharmacy, Alexandria. The animals were divided into groups, **4** rats each. A group of animals received the vehicle and served as a control. Another group received a standard dose of estradiol (0.09 µmol/rat/day). The compounds were administered subcutaneously once daily over a 4-day period in 0.1 ml DMSO (0.09 mmol/day/rat). The rats were weighed 24h after the last dose and vaginal smears were taken and examined under the microscope. The animals were then sacrified and the uteri were carefully dissected out, freed from fat and connective tissue, blotted, weighed, dried at 60 °C for 24h and weighed again. Table 2 shows the gain in uterine weight calculated as mg uterine weight/100 g body weight and agonistic activity (%) estimated by the following formula[6]:

$$\% \text{ Agonistic activity} = \frac{(W_T - W_V)}{(W_S - W_V)} \times 100$$

Where:
W_T = relative dry uterine weight of animals treated with test compound.
W_S = relative dry uterine weight of animals treated with a standard dose of estadiol (0.09 µmol/rat/day).
W_V = relative dry uterine weight of control animals.

The previous procedure was repeated using different doses of compound **26** (0.0225, 0.045, 0.09, 0.18 µmol/rat/day).

Antiuterotrophic Activity

The antiuterotrophic activity[25,26] of the synthesized compounds **3–30** was assessed in immature albeno female rats (approximately 45–55 g, 21–23 days of age). The compounds were administered subcutaneously in 0.1 ml DMSO along with 0.09 µmol/rat/day of estradiol (in 0.1 ml DMSO) at 2 different sites for 3 consecutive days. Inhibition was expressed as percent inhibition from the formula of Hartmann et al.[6,26] (Table 2).
Where:

$$\% \text{ Inhibition} = 100 - \left[\frac{W_{S,T} - W_V}{W_S - W_V} \times 100\right]$$

$W_{S,T}$ = relative dry uterine weight of animals treated with estradiol + test compound.

The previous procedure was repeated using differeent doses of compound **12** (0.0225, 0.045, 0.09, 0.18 µmol/rat/day).

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Received: May 30, 1997 [FP224]