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Synthesis, binding affinities and uterotrophic activity of some 2-substituted estradiol and ring-A-fused pyrone derivatives

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Summary — A series of estradiol analogs has been synthesized and examined as potential estrogens. Nuclear modifications included a variety of substituents at the 2 position of estradiol, which was previously thought to be inhibitory for activity, and inclusion of the 3-phenolic hydroxyl group in a γ-pyrone and 3'-formylchromone rings fused to ring A of estradiol. The estrogen relative binding affinities and *in vivo* assays for uterotrophic activity in rats showed that all the tested compounds were capable of displacing $[^3H]E_2$ from the estrogen receptor sites by different degrees. The highest inhibition of $[^3H]E_2$ binding (78%) to the estrogen receptor was displayed by 2-acetylestradiol which was also a potent uterotrophic agent. Omission of the free 3-hydroxyl functionality by inclusion in a γ-pyrone ring produced a chromone derivative that was capable of inhibiting $[^3H]E_2$ binding by 60% and displayed a uterotrophic response of 97%. Further nuclear modification by introduction of thiosemicarbazone moieties decreased the uterotrophic activity, the highest activity being elicited by the *p*-bromophenyl thiosemicarbazone derivative. The diketone 2-benzoylacetylestradiol 17β-acetate, 2-(3'-benzylideneacetyl)estradiol and 2-[3'-(3-anisylidene)acetyl]estradiol exhibited high inhibition of binding affinity while eliciting ≈ 50% the uterotrophic activity of estradiol.

2-acetylestradiol 17β-acetate / 3'-formylestradiolchromone / thiosemicarbazones / diketone / chalcone derivatives / 2-pyrazoloestradiol / 2-oxazoloestradiol / estradiolpyranone / binding affinity / uterotrophic activity

Introduction

The estrogen receptor, which elicits a uterotrophic response, has been used in studies defining the relationship between binding affinity, structure and hormonal activity [1, 2]. These studies have suggested that estrogens must have 17β-hydroxyl and phenolic 3-hydroxyl groups in order to possess high binding affinity and, consequently, potent hormonal activity. Different descriptions of the requisite dispositions of these 2 oxygens have been reported [3, 4]. However, the effects of different numbers of oxygens are still unpredictable. In this regard, it was surprising that reports appeared showing that the absence of the normally requisite oxygens for steroids can prove beneficial. In the field of estrogens, Kincl and Dorfman [5] found that, relative to mestranol, the 3-desoxy derivative 17α-ethynylestra-1,3,5(10)-trien-17-ol had 50% of the oral postcoital antifertility activity in rats and only 5% of the oral uterotrophic activity in mice, and that the 17-desoxy compound estra-1,3,5(10)-trien-3-ol had 15% of the oral postcoital antifertility activity in rats and 0.2% of the oral uterotrophic activity in mice. Although similar 17-desoxy estrones were synthesized by Krubiner *et al* [6], the estrogenic activity was not evaluated. Recently, other 17-desoxy estrogens, in which the oxygen was replaced by other heteroatoms or 17-alkyl substituents showing different degrees of unsaturation and/or numbers of heteroatoms, were prepared and examined for a separation of uterotrophic and antifertility activity [7]. In another report, the antifertility and estrogenicity of 3-desoxyethinylestradiol [8] were simultaneously reduced in magnitude. In contrast, the corresponding silylated 3-desoxyethinylestradiol analogue retained equal antifertility potency to that of ethinylestradiol but with reduced estrogenicity.

Following this rationale, our aim was to further explore these effects in a series of estradiol deriva-

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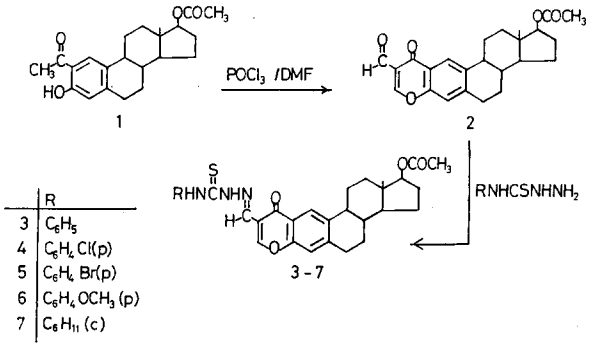
tives in which the 3-oxygen functionality, although still present, is included in a γ-pyrone ring structure with the intention of altering the steroidal nucleus to determine the relationship between such a structural modification, affinity and the degree of activity. The aldehydic group in the 3-position of the γ-pyrone ring, by virtue of its high polarity, would help in accommodating the products at the receptor site. On the other hand, conversion of the aldehydic carbonyl into various thiosemicarbazone moieties was meant to investigate the effect of changing the polarity and bulkiness of such groups on the activities in question.

Moreover, C-2 substitution has proved to be an intriguing system from the viewpoint of both biological interest and synthetic challenge, particularly in the study of its effect on subsequent side-chain modifications. The high order of estrogenic activity possessed by some of these compounds was found to be surprisingly beneficial [9], and indicated that less demanding molecular requirements are needed at the receptor sites and that the role of the C-2 functionality in estrogens had not been fully evaluated previously. The preparation of a series of substituted chalcones, and diketone, oxazole and pyrazole ring systems derived from 2-acetylestradiol has therefore been described. A study of the structure–activity relationships of these 2-substituted estrogens was also undertaken to evaluate biological activity among a series of related compounds in which the 2-acetyl group was converted into different moieties.

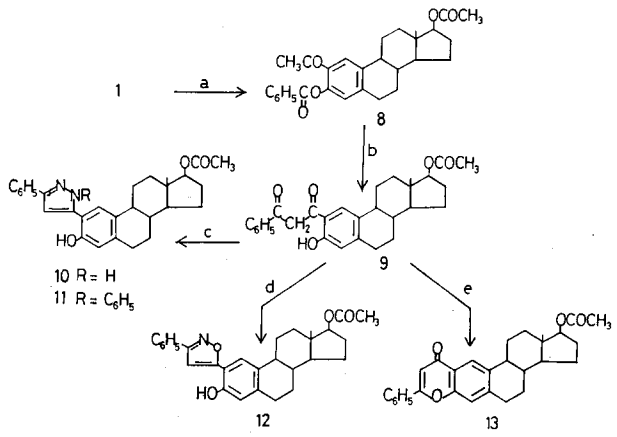
Chemistry

The products under consideration were synthesized according to schemes 1–3.

The synthesis of 2-acetylestradiol 17β-acetate **1** [10] was accomplished by treating estradiol with

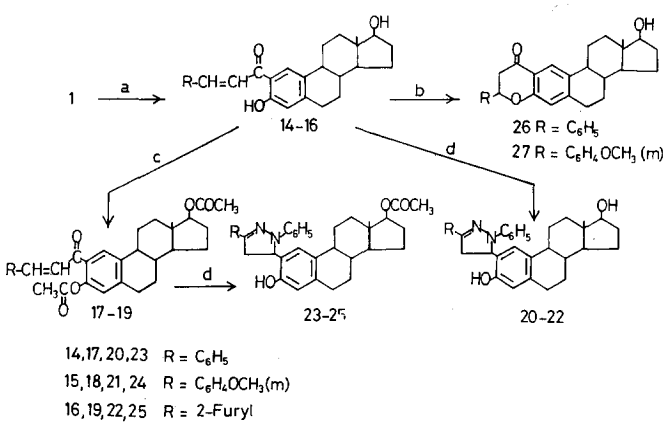


Scheme 1. 3'-Formylestradiolchromone and N^4-substituted thiosemicarbazones.



Scheme 2. 2-Substituted estradiol and ring-A-fused pyrone derivatives. **a**. Benzoyl chloride/dry pyridine/0°C; **b**. KOH/ dry pyridine/50°C; **c**. hydrazine hydrate/ethanol/glacial acetic acid or phenylhydrazine/glacial acetic acid or phenylhydrazine hydrochloride/pyridine; **d**. hydroxylamine hydrochloride/ pyridine or hydroxylamine hydrochloride/ethanol/ sodium acetate; **e**. phenylhydrazine hydrochloride/ glacial acetic acid or conc HCl/glacial acetic acid.

acetyl chloride under Friedel–Crafts reaction conditions. The mixture of compound **1**, dimethylformamide and phosphorus oxychloride was allowed to react under Vilsmeier–Haack reaction conditions [11, 12] at –10°C and left overnight at rt for completion of the reaction. The reaction proceeded through the double formylation of the methyl group by the Vilsmeier reagent [12] and afforded a good yield of the chromone derivative **2**. The structure of the steroidal chromone **2** was confirmed by elemental analysis, IR, UV and ^{1}H-NMR spectra. The IR spectrum indicated the absence of the 3-hydroxyl function of the starting 2-acetylestradiol 17β-acetate **1** but did show bands due to the carbonyl groups of the 17β-acetoxy moiety and the γ-pyrone ring and an absorption band for the aldehydic carbonyl function. The UV spectrum indicated a bathochromic shift relative to compound **1** due to extended conjugation of the double bond of the γ-pyrone ring with the aromatic ring A of estradiol. The ^{1}H-NMR spectrum lacked the signals for the 2-acetyl and the 3-hydroxyl protons of the starting material. A new singlet at 8.55 ppm was assigned to the 2'-H of the γ-pyrone ring and another at 10.40 ppm was assigned to the aldehydic proton. The aldehydic group of 3'-formylestradiolchromone **2** was then reacted with the equivalent amount of the appropriately substituted thiosemicarbazides in boiling ethanol. The products **3–7** always separated out in an almost pure form.



Scheme 3. 2-Substituted estradiol and ring-A-fused pyrone derivatives. **a.** RCHO/10% NaOH in ethanol/rt or 40% aqueous KOH; **b.** NaOH in methanol, pH 10/rt; **c.** acetic anhydride/dry pyridine; **d.** phenylhydrazine/ethanol/Δ.

To carry out the Baker–Venkataraman rearrangement [13, 14], the most straightforward preparative method in this study appeared to be the benzoylation of 2-acetylestradiol **1** with benzoyl chloride in dry pyridine [15], followed by treatment of the 3-benzoate derivative **8** with previously dried, pulverized potassium hydroxide in hot dry pyridine [16] to give the required diketone **9**. The ^{1}H-NMR spectrum of compound **9** showed, in addition to the expected signals, 2 new singlets at 4.60 ppm assigned to the methylenic proton of the keto tautomer and at 6.73 ppm assigned to the vinylic proton of the enol tautomer, indicating that the diketone **9** exists in an equilibrium between the 2 forms. The pyrazolo and isoxazolo derivatives **10** and **12** were obtained by condensation of the steroidal diketone **9** with hydrazine hydrate in boiling acidified ethanol and with hydroxylamine in pyridine, respectively. Depending on the reaction conditions, condensation of the diketone **9** with phenylhydrazine hydrochloride in pyridine gave the required pyrazolo derivative **11** *(Method A)*, while in glacial acetic acid, the estradiolchromone **13** was obtained as evidenced by superimposability with authentic IR, UV, ^{1}H-NMR spectra and mixed mp with a sample of **13** prepared by cyclizing the diketone with phenylhydrazine in hot glacial acetic acid containing 3 drops of conc HCl. Repeating the reaction with phenylhydrazine only in glacial acetic acid *(Method B)* produced the diphenylpyrazole **11** in a relatively higher yield. This demonstrated that in the absence of HCl, phenylhydrazine conducted cyclization into the diphenylpyrazole **11** without competition for the chromone formation.

The chalcone derivatives **14–16** were prepared by condensing **1** with different aldehydes in 10% ethanolic NaOH, with an improved yield when 40% aqueous KOH was used. Application of the same procedure to obtain the corresponding *p*-nitro, *p*-dimethylamino- and *p*-hydroxychalcone derivatives was fruitless. Cyclization of the chalcones **14–16** with phenylhydrazine in ethanol produced the pyrazolino derivatives **20–22** in low yields. The yields were improved by using the chalcone diacetate derivatives **17–19**. However, under these conditions, the 3-acetoxy function was hydrolyzed during the reaction. Ring closure of the chalcones **14** and **15** into the corresponding dihydro-γ-pyrone derivatives **26** and **27** was accomplished with methanolic NaOH at pH 10. Application of the same conditions for the preparation of the 2'-furyldihydropyrone produced a mixture of the chalcone and the dihydropyrone derivatives which could not be separated.

Results and discussion

Effect on binding affinity

The binding affinity data indicated that all the tested compounds were capable of displacing [^{3}H]E_2 from the estrogen receptor sites to different degrees (table I). The highest binding affinity (75%) to the estrogen receptor was observed with the parent compound 2-acetylestradiol **1**. Inclusion of the 3-hydroxyl group in a γ-pyrone structure gave a compound with good binding affinity to the estrogen receptor (60%). Conversion of the aldehydic side chain in 3'-formylestradiolchromone **2** into thiosemicarbazone moieties did not produce a significant reduction in binding affinity. The N^4-cyclohexylthiosemicarbazone derivative **7** displayed the lowest ability (51%) to inhibit the binding of [^{3}H]E_2 to estrogen receptor sites. This demonstrated the steric effect induced by the puckered cyclohexyl function with its bulky spatial arrangement compared with those of phenyl or substituted phenyl group.

The diketone **9** and the chalcones **14** and **15** suppressed the *in vitro* binding of estradiol by 72, 75 and 72% respectively at a concentration of 2.5 nM. They were more potent than the cyclized compounds containing a heterocyclic ring at the 2-position of the estradiol nucleus. The 2-pyrazolo- and 2-isoxazoloestradiol derivatives **10–12** produced 59, 60 and 63% inhibition of the binding affinity respectively at a concentration of 2.5 nM.

Table I. Inhibition of $[^3H]E_2$ binding to the estrogen receptor of immature rat uterine nuclei.

Comp No	*% Inhibition of $[^3H]E_2$ binding to estrogen receptor*[a]
1	75
2	60
3	58
4	59
5	56
6	55
7	51
8	57
9	72
10	59
11	60
12	63
13	50
14	75
15	72
26	57

[a]At a concentration of 2.5 nM.

Effect on uterotrophic activity

At a dose of 0.09 μmol/d/rat, 2-acetylestradiol 17β-acetate **1** was, as recently reported [9], more potent than estradiol as an estrogen and showed the highest uterotrophic activity (110%) relative to estradiol activity when administered sc (table II). Conversion of **1** into 3'-formylestradiolchromone **2** produced a compound whose uterotrophic activity was retained compared with estradiol (97%). The *p*-bromophenyl thiosemicarbazone derivative **5** showed a uterotrophic response of 90% of the estradiol activity. A uterotrophic activity of 66% was observed for the phenyl derivative **3**. The other thiosemicarbazone derivatives showed moderate estrogenic activity.

Although the diketone **9** and the chalcone derivatives **14** and **15** exhibit high relative binding affinities, they initiated estrogenic responses of only ≈ 50% that produced by estradiol. However, as a result of weaker interactions with the estrogen receptor, the 2-pyrazolo and 2-oxazolo derivatives **10–12** showed weaker uterotrophic activity of 15–16%. A sharp drop in both the relative binding affinity and the uterotrophic activity was observed upon inclusion of the 3-hydroxyl group in a γ-pyrone ring structure (compounds **13** and **26**). Benzoylation of the free 3-hydroxyl group as in compound **8** reduced the percentage inhibition of the tritiated estradiol binding to the receptor. However, a uterotrophic activity of 56% was observed, suggesting that partial hydrolysis may have occurred in the rat.

Conclusions

The fact that our studies show that 3'-formylestradiolchromone **2** has good binding affinity to the estrogen receptor sites while still eliciting high uterotrophic activity indicates that the presence of a free C-3 oxygen function on the estradiol skeleton, which was previously thought to be a requisite for activity, is not essential for receptor binding and does not affect the estrogenic potency profoundly. Our findings have also suggested that high uterotrophic activity in the rat, despite moderate receptor binding affinity for compound **5**, may be due to its transformation into more active metabolites which are responsible for the high uterotrophic activity.

The data obtained have also enabled us to make a preliminary assessment of the potential selectivity of C-2 substituents in steroidal compounds. Our results have established evidence of the good affinity of some C-2-substituted estradiol derivatives and emphasized the benefit of such structural modifications in this position to biological potency. These results are quite encouraging and provide a background for future studies on the role of C-2 substitution in the action of estrogenic steroids.

Experimental protocols

Chemistry

Melting points were recorded in open capillaries on a Griffin melting point apparatus and are uncorrected. UV spectra were recorded on a Perkin–Elmer Model 5509 spectrophotometer. IR spectra were determined in nujol mulls on a Beckman 1410 spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian A-60 spectrometer with tetramethylsilane as internal standard. Values are given in ppm (δ) (s, singlet; d, doublet; dist, distorted; t, triplet; dd, doublet of doublets; m, multiplet and br, broad). MS was performed on a Dupont/Bell mass spectrometer. The homogeneity of the products was checked by ascending TLC on silica-gel-coated glass plates visualized by iodine vapor. Microanalytical data are indicated by the symbols of the elements and were within ±0.4% of theoretical values. The elemental analyses were performed by the Microanalytical Unit, Faculty of Science, Cairo University, Egypt. Tritiated estradiol ($[^3H]E_2$), spec act 49.3 ci/mol, was purchased from New England Nuclear, Boston, MA, USA. Radioactivity was counted in a Packard Tri-carb liquid scintillation spectrometer (Searle Mark III, 6880), USA.

3'-Formylestradiolchromone 17β-acetate 2

Phosphorus oxychloride (25 ml) was added dropwise over 10 min to a well-stirred and cooled (–10°C) solution of 2-acetylestradiol 17β-acetate [10, 17, 18] **1** (0.5 g, 1 mmol) in dry dimethylformamide (30 ml). The mixture was warmed to 20°C and the yellowish-brown viscous solution obtained was stirred overnight at rt and poured onto crushed ice. The yellow precipitate that formed was collected by filtration, washed with water and dried. Crystallization from a benzene/light petroleum (60–80°C) solution gave compound **2** as yellow crystals in

Table II. Estrogenic potencies of the synthesized estradiol derivatives.

Comp. N°.	Dose (mg/kg) body weight	mg uterine weight	Wet uterine weight/100 g body weight	% Dry weight/wet weight (n)[a]	Uterotrophic activity %
Control[b]	--	26.60 ± 3.18	27.24 ± 2.57	22.00 ± 0.90 (5)	--
Estradiol	0.25	165.50 ±11.07***	167.80 ± 8.50***	20.77 ± 0.30 (4)	100
1	0.32	182.00 ± 16.20***	187.08 ± 11.69***	20.82 ± 0.15 (5)	110
2	0.36	161.00 ± 10.10***	160.71 ± 6.59***	20.58 ± 0.03 (4)	97
3	0.49	109.00 ± 6.50***	112.90 ± 6.14***	22.49 ± 0.74 (5)	66
4	0.53	95.60 ± 4.38**	97.20 ± 6.80**	22.78 ± 0.10 (5)	57
5	0.57	148.75 ± 17.30***	102.00 ± 7.90***	21.36 ± 0.17 (4)	90
6	0.52	97.00 ± 8.30**	98.30 ± 5.83**	22.75 ± 0.23 (5)	59
7	0.50	80.00 ± 8.20**	82.50 ± 7.04**	21.16 ± 0.36 (4)	48
Control[b]	--	14.00 ± 2.59	22.90 ± 2.60	24 ± 0.20 (5)	--
Estradiol	0.25	92.80 ± 7.02***	178.14 ± 8.60***	23 ± 1.50 (5)	100
8	0.42	60.50 ± 0.90**	99.40 ± 1.66**	24 ± 1.20 (5)	56
9	0.42	53.50 ± 1.70**	86.70 ± 1.79**	24 ± 0.40 (5)	46
10	0.42	15.80 ± 2.04	26.06 ± 2.14	25 ± 1.20 (5)	15
11	0.48	16.80 ± 2.62	28.29 ± 4.30	24 ± 0.23 (5)	16
12	0.42	18.00 ± 3.86	31.30 ± 7.50	23 ± 0.92 (5)	18
13	0.406	17.60 ± 1.29	27.50 ± 3.74	26 ± 2.30 (5)	15
14	0.37	46.60 ± 2.66**	77.28 ± 8.67**	26 ± 1.15 (5)	43
15	0.38	49.00 ± 4.52**	80.47 ± 7.09**	24 ± 0.32 (5)	45
16	0.37	16.00 ± 1.64	27.76 ± 2.18	24 ± 0.40 (4)	16

[a]Number of animals indicated in parentheses; [b]rats received vehicle (olive oil) and served as control; the results were expressed as mean ± SEM; data were analyzed by one-way variance; Student's *t*-test for unpaired observations was used; differences between means were considered significant if $P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

64% yield, mp: 230–232°C. Anal $C_{24}H_{26}O_5$ (C, H). UV $\lambda_{max}^{methanol}$: 205 (4.48), 225 (4.43), 308 (4.02) and 342 (3.98) nm (log ε). IR: ν 1730 (C=O, C-17-acetate), 1690 (CHO), 1645 (C=O, γ-pyrone), 1620, 1580 (C=C), 1245 and 1180 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$): δ 0.83 (s, 3H, 18-CH_3), 2.08 (s, 3H, C-17-$OCOCH_3$), 4.60 (t, dist, 1H, C-17-α-H), 7.30 (s, 1H, C-4-H), 8.20 (s, 1H, C-1-H), 8.51 (s, 1H, pyrone-2'-H) and 10.77 (s, 1 H, CHO).

Estradiolchromone 17β-acetate 3'-formylthiosemicarbazones 3–7
A solution of the appropriate N^4-substituted-3-thiosemicarbazide (0.3 mmol) in ethanol (10–20 ml) and 3 drops glacial acetic acid were added to a solution of the 3'-formylestradiolchromone **2** (100 mg, 0.2 mmol) in chloroform (5 ml). The mixture was heated under reflux for 6 h and left at rt overnight. The deposited products are insoluble in the ordinary crystallization solvents and were purified by filtration from boiling ethanol. IR for compounds **3–7**: ν 3320–3280, 3240 (NH), 1730 (C=O, C-17-acetate), 1645 (C=O, γ-pyrone), 1630–1620 (C=N), 1600–1550 (C=C), 1530–1500, 1335–1320, 1180–1150 and 940–910 cm^{-1} (NCS amide I, II, III and IV bands).

Compound 3. This was obtained in 77% yield, mp: 242–245°C. Anal $C_{31}H_{33}N_3O_4S$ (C, H, N, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 0.81 (s, 3H, 18-CH_3), 2.01 (s, 3H, C-17-$OCOCH_3$), 4.67 (m, 1 H, 17α-H), 7.10–8.30 (m, 7H, 5 Ar-H + C-1-H + C-4-H), 8.50 (s, 1H, pyrone-2'-H), 9.23 (s, 1H, N=CH), 10.00 (s, 1H, N-H disappears on deuteration) and 11.80 (s, 1H, NH disappears on deuteration).

Compound 4. This was obtained in 86% yield, mp: 248–250°C. Anal $C_{31}H_{32}ClN_3O_4S$ (C, H, N, S, Cl). MS (M^+ 409).

Compound 5. This was obtained in 70% yield, mp: 210–212°C. Anal $C_{31}H_{32}BrN_3O_4S$ (C, H, N, S, Br).

Compound 6. This was obtained in 69% yield, mp: 205–207°C. Anal $C_{32}H_{35}N_3O_5S$ (C, H, N, S).

Compound 7. This was obtained in 65% yield, mp: 250–252°C. Anal $C_{31}H_{39}N_3O_4S$ (C, H, N, S).

2-Acetylestradiol-3-benzoate 17β-acetate 8
Benzoyl chloride (4 ml, 23 mmol) was added dropwise to a solution of 2-acetylestradiol 17β-acetate **1** [5] (2 g, 5 mmol) in

dry pyridine cooled in an ice bath. The mixture was then stirred at rt for 14 h, poured onto crushed ice and acidified with conc HCl. The mixture was extracted with benzene (3 x 20 ml) and the extracts were washed twice with water and dried (anhydrous Na_2SO_4). The solvent was evaporated *in vacuo* and the residue treated with ether to separate the product **8** which was crystallized from ethanol, yield 1.6 g (79%), mp: 158–160°C. Anal $C_{29}H_{32}O_5$ (C, H). IR: ν 1730 (C=O, C-17-acetate), 1680 (C=O, C-3-acetate), 1625 (C=O, C-2-acetyl), 1580, 1485 (C=C aromatic), 1265 and 1060 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$): δ 0.88 (s, 3H, 18-CH_3), 2.10 (s, 3H, C-17-OCOCH_3), 2.50 (s, 3H, C-2-COCH_3), 4.60–4.80 (m, 1H, C-17α-H), 6.90 (s, 1H, C-4-H), 7.50–7.60 (m, 3H, Ar-H), 7.85 (s, 1H, C-1-H), 8.18 and 8.30 (2 d, dist, 2H, Ar-H *ortho* to C=O).

2-Benzoylacetylestradiol 17β-acetate ***9***

A solution of **8** (0.5 g, 1 mmol) in dry pyridine (7 ml) at 50°C was treated with pulverized KOH (previously heated at 100°C for 30 min) (50 mg, 1 mmol) added in small portions and stirred at this temperature for 1 h. The solution was poured onto stirred 10% acetic acid (20 ml) to separate an oily yellow product which was extracted with ether. The extracts were washed with water, dried (anhydrous Na_2SO_4) and evaporated to dryness. The residue was treated with light petroleum (60–80°C) to deposit the product **9** which was filtered, dried and crystallized from ethanol. Yield 0.4 g (80%), mp: 170–172°C. Anal $C_{29}H_{32}O_5$ (C, H). UV $\lambda_{max}^{methanol}$: 225 (4.6), 355 (4.3) and 370 (3.32) nm (log ε). IR: ν 3450 (OH), 1730 (C=O, C-17-acetate), 1680 and 1640 (C=O), 1580, 1500 (C=C aromatic), 1250 and 1080 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$): δ 0.85 (s, 3H, 18-CH_3), 2.08 (s, 3H, C-17-OCOCH_3), 4.60 (s, 2H, $COCH_2CO$), 4.70 (t, 1H, C-17α-H), 6.80 (s, 1H, C-4-H), 7.50 (m, dist, 3H, Ar-H), 7.67 (s, 1H, C-1-H), 7.90 and 8.00 (2d, dist, 2H, Ar-H *ortho* to C=O) and 11.88 (s, 1H, C-3-OH exchangeable with D_2O).

2-(3'-Phenylpyrazol-5'-yl)estradiol 17β-acetate ***10***

A solution of hydrazine hydrate 99% (0.5 ml, 10 mmol) in ethanol (1 ml) was added to a solution of the diketone **9** (230 mg, 0.5 mmol) in ethanol (20 ml) containing 3 drops glacial acetic acid. The mixture was heated under reflux for 2 h and concentrated. The product deposited on cooling to rt was filtered, dried and crystallized from ethanol. Yield 100 mg (53%), mp: 280–282°C. Anal $C_{29}H_{32}N_2O_3$ (C, H, N). UV $\lambda_{max}^{methanol}$: 222 (4.4), 255 (4.25), 265 (3.82) and 305 (4.2) nm (log ε). IR: ν 3440 (OH), 3200 (NH), 1730 (C=O, C-17-acetate), 1610 (C=N), 1280 and 1080 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 0.72 (s, 3H, 18-CH_3), 2.02 (s, 3H, C-17-OCOCH_3), 4.60–4.70 (m, 1H, C-17α-H), 6.65 (s, 2H, C-4-H + pyrazole-4'-H), 7.30–7.60 (m, 3H, Ar-H), 7.65 (s, 1H, C-1-H), 7.85 (d, 2H, Ar-H *ortho* to pyrazole), 10.69 (s, 1H, OH exchangeable with D_2O), 12.80 and 13.50 (2s, 1H, 2 isomeric pyrazole-1'-H).

2-(1',3'-Diphenylpyrazol-5'-yl)estradiol 17β-acetate ***11***

Method A. A solution of phenylhydrazine hydrochloride (145 mg, 1 mmol) and the diketone **9** (230 mg, 0.5 mmol) in pyridine (10 ml) was heated under reflux for 6 h. The mixture was poured into ice-cold water to give compound **11** as yellow crystals which were filtered, washed with water, dried and crystallized from ethanol. Yield 150 mg (56%), mp: 295–297°C. Anal $C_{35}H_{36}N_2O_3$ (C, H, N). UV $\lambda_{max}^{methanol}$: 223 (4.2), 252 (4.12), 270 (3.92) and 289 (4.4) nm (log ε). IR: ν 3450 (OH), 1730 (C=O, C-17-acetate), 1625 (C=N), 1280 and 1120 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H, 18-CH_3), 2.04 (s, 3H, C-17-OCOCH_3), 4.70 (m, 1H, C-17α-H), 6.62 (s, 1H, C-4-H), 6.80 (s, 1H, pyrazole-4'-H), 7.20–7.45 (m, 6H, Ar-H), 7.50 (s, 1H, C-1-H), 7.75–8.00 (m, 4H, Ar-H) and 10.90 (s, br, 1H, OH exchangeable with D_2O).

Method B. Phenylhydrazine (0.5 ml, 0.4 mmol) was added to a solution of the diketone **9** (100 mg, 0.2 mmol) in glacial acetic acid (5 ml) and the mixture heated under reflux for 3 h. Workup of the mixture as described in *Method A* gave 80 mg of compound **11** (59%).

2-(3'-Phenylisoxazol-5'-yl)estradiol 17β-acetate ***12***

Method A. A solution of hydroxylamine hydrochloride (35 mg, 0.5 mmol) and the diketone **9** (230 mg, 0.5 mmol) in pyridine (10 ml) was heated under reflux for 12 h. The white crystals that deposited on cooling the mixture to room temperature were filtered, dried and crystallized from dioxane/water. Yield 120 mg (53%), mp: 280–282°C. Anal $C_{29}H_{31}NO_4$ (C, H, N). UV $\lambda_{max}^{methanol}$: 270 (2.479) and 313 (1.73) nm (log ε). IR: ν 3500–3350 (OH), 1730 (C=O, C-17-acetate), 1615 (C=N), 1280 and 1120 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 0.69 (s, 3H, 18-CH_3), 2.07 (s, 3H, C-17-OCOCH_3), 4.52 (m, 1H, C-17α-H), 6.72 (s, 1H, C-4-H), 7.23 (s, 1H, isoxazole-4'-H), 7.50 (m, 3H, Ar-H), 7.66 (s, 1H, C-1-H) and 7.59 (m, 2H, Ar-H).

Method B. The same procedure as in *Method A* was carried out but using a few crystals of anhydrous sodium acetate in ethanol (15 ml) instead of pyridine. The product separated in 85% yield.

2'-Phenylestradiolpyran-4'-one 17β-acetate ***13***

Method A. A solution of the diketone **9** (115 mg, 0.25 mmol) in glacial acetic acid (5 ml) containing 3 drops conc HCl was heated under reflux for 1 h. The mixture was diluted with ethyl acetate, washed twice with saturated sodium bicarbonate solution, then brine and water, and dried (anhydrous Na_2SO_4). The solvent was evaporated to dryness and the residue obtained was crystallized from ethanol. Yield 80 mg (70%), mp: 270–272°C. Anal $C_{29}H_{30}O_4$ (C, H). UV $\lambda_{max}^{methanol}$: 220 (3.95), 252 (4.39) and 302 (4.38) nm (log ε). IR: ν 1730 (C=O, C-17-acetate), 1645 (C=O, γ-pyrone), 1580, 1490 (C=C aromatic), 1250 and 1080 cm^{-1} (C-O-C). ^{1}H-NMR ($CDCl_3$): δ 0.86 (s, 3H, 18-CH_3), 2.10 (s, 3H, C-17-OCOCH_3), 4.70 (t, 1H, C-17α-H), 6.82 (s, 1H, pyran-3'-H), 7.30 (s, 1H, C-4-H), 7.55 (m, 2H, Ar-H), 7.98 (m, 2H, Ar-H) and 8.12 (s, 1H, C-1-H). MS (M^+ 442).

Method B. A solution of equimolar amounts of the diketone **9** (230 mg, 0.5 mmol) and phenylhydrazine hydrochloride in glacial acetic acid (10 ml) was heated under reflux for 2 h. After cooling, the mixture was poured into ice-cold water and the precipitate washed with water, dried and crystallized from ethanol. Yield 170 mg (65%), mp: 271–272°C.

2-[3'-Arylidene or 3'-(2-furylidene)acetyl]estradiol ***14–16***

Method A. A solution of compound **1** (0.5 g, 1.4 mmol) and the selected aldehyde (1.8 mmol) in 10% ethanolic NaOH (10 ml) was stirred at rt for 5 h. The mixture was made acidic with 10% aqueous HCl. The yellow crystals obtained were filtered, washed with water, dried and crystallized from the appropriate solvent.

Method B. An ice-cold solution of compound **1** (0.5 g, 1.4 mmol) and the appropriate aldehyde (1.8 mmol) in ethanol (20 ml) was treated dropwise with 40% aqueous KOH (10 ml). The mixture was left overnight at rt and poured onto crushed ice containing 10 ml conc HCl. The precipitate was filtered, washed with water and dried. IR for compounds **14–16**: ν 3600–3400 (OH), 1635 (C=O) and 1600 cm^{-1} (C=C aromatic).

2-(3'-Benzylideneacetyl)estradiol ***14***. This was crystallized from aqueous ethanol in 30% *(Method A)* and 75% *(Method B)* yields, mp: 175–176°C. Anal $C_{27}H_{30}O_3$ (C, H). UV $\lambda_{max}^{methanol}$: 315 (4.46) and 360 (4.04) nm (log ε). ^{1}H-NMR ($CDCl_3$): δ 0.83 (s, 3H, 18-CH_3), 3.76 (t, 1H, C-17α-H), 6.78 (s, 1H, C 4-H), 7.79 (s, 1H, C-1-H), 7.65 (d, 1H, *J* = 14 Hz, CHCO), 7.91 (d, 1H, *J* = 14 Hz, RCH=) and 7.30–7.70 (m, 5H, Ar-H).

2-(3'-m-Anisylideneacetyl)estradiol ***15***. This was crystallized from ether/light petroleum in 35% *(Method A)* and 80% *(Method B)* yields, mp: 170–172°C. Anal $C_{28}H_{32}O_4$ (C, H). UV $\lambda_{max}^{methanol}$: 313 (4.5) and 358 (4.02) nm (log ε). ^{1}H-NMR ($CDCl_3$): δ 0.84 (s, 3H, 18-CH_3), 3.82 (m, 1H, C-17α-H), 3.85 (s, 3H, OCH_3), 6.75 (s, 1H, C-4-H), 7.20–8.00 (m, 4H, Ar-H), 7.60 (s, 1H, C-1-H), 7.70 (d, 1H, *J* = 14 Hz, CH-CO), 7.89 (d, 1H, *J* = 14 Hz, RCH=).

2-[(3'-Furylidene)acetyl]estradiol ***16***. This was purified by chromatography on a silica-gel column (Kieselgel 100 E Merck, 70-30 mesh ASTM) using benzene/light petroleum (60–80°C) (80:20 v/v) as eluent, yield 20% *(Method A)* or 53% *(Method B)*, mp: 150–152°C. Anal $C_{25}H_{28}O_4$ (C, H). UV: $\lambda_{max}^{methanol}$: 340 (4.52) and 370 (4.04) nm (log ε). ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H, 18-CH_3), 3.70 (m, 1H, C-17α-H), 6.20 (m, 1H, furyl-4-H), 6.50 (d, 1H, *J* = 3 Hz, furyl-3-H), 6.64 (s, 1H, C-4-H), 7.62 (s, 1H, C-1-H), 7.10 (d, 1H, *J* = 14 Hz, CHCO), 7.37 (d, 1H, *J* = 14 Hz, RCH=), 7.50 (d, 1H, *J* = 3 Hz, furyl-5-H).

2-[(3'-Arylidene or 3'-(2-furylidene)acetyl]estradiol 3,17β-diacetates ***17–19***
A solution of compounds **14–16** in a mixture of dry pyridine (5 ml) and acetic anhydride (5 ml) was heated under reflux for 1 h. After cooling to rt, the mixture was poured into ice-cold water and the product was filtered, washed with water, dried and crystallized from aqueous ethanol. IR for compounds **14–16**: ν 1730 (C=O, C-17-acetate), 1680 (C=O, C-3-acetate), 1660 (C=O), 1600 (C=C aromatic), 1250 and 1070 cm^{-1} (C-O-C).

2-(3'-Benzylideneacetyl)estradiol 3,17β-diacetate ***17***. This was obtained in 75% yield, mp: 196–198°C. Anal $C_{31}H_{34}O_5$ (C, H). UV $\lambda_{max}^{methanol}$: 319 (4.5) and 365 (4.6) nm (log ε).

2-(3'-m-Anisylideneacetyl)estradiol 3,17β-diacetate ***18***. This was obtained in 82% yield, mp: 201–203°C. Anal $C_{32}H_{36}O_6$ (C, H). UV $\lambda_{max}^{methanol}$: 315 (4.7) and 359 (4.1) nm (log ε).

2-[3'-(2-Furylideneacetyl)]estradiol 3,17β-diacetate ***19***. This was obtained in 74% yield, mp: 183–185°C. Anal $C_{29}H_{32}O_6$ (C, H). UV $\lambda_{max}^{methanol}$: 339 (4.6) and 373 (4.05) nm (log ε). ^{1}H-NMR ($CDCl_3$): δ 0.81 (s, 3H, 18-CH_3), 2.02 (s, 3H, C-17-$OCOCH_3$), 2.07 (s, 3H, C-3-$OCOCH_3$), 4.57 (t, 1H, C-17α-H), 6.23 (m, 1H, furyl-4-H), 6.50 (d, 1H, *J* = 3 Hz, furyl-3-H), 6.60 (s, 1H, C-4-H), 7.10 (d, 1H, *J* = 3 Hz, CH-CO), 7.38 (d, 1H, *J* = 3 Hz, RCH=), 7.50 (d, 1H, *J* = 3 Hz, furyl-5-H), 7.62 (s, 1H, C-1-H).

2-[3'-Aryl or 3'-(2-furyl)-4',5'-dihydro-1'-phenylpyrazol-5'-yl] estradiol ***20–22*** *and 17β-acetates* ***23–25***
A solution of compounds **14–19** (0.2 mmol) in ethanol (10 ml) was treated with phenylhydrazine (0.4 mmol), heated under reflux for 6-8 h, concentrated and left overnight at rt for complete deposition of the products. Compounds **20–25** were filtered, dried and crystallized from ethanol or aqueous ethanol. IR for compounds **20–25**: ν 3600–3350 (OH), 1725 (C=O, C-17-acetate), 1600 and 1495 cm^{-1} (C=N and C=C aromatic).

2-(3'-Phenyl-4',5'-dihydro-1'-phenylpyrazol-5'-yl)estradiol ***20***. This was obtained in 40% yield, mp: 165–167°C. Anal $C_{33}H_{36}N_2O_2$ (C, H, N). UV $\lambda_{max}^{methanol}$: 260 (4.2) and 300 (3.5) nm (log ε). ^{1}H-NMR ($CDCl_3$): δ 0.76 (s, 3H, 18-CH_3), 2.95–3.10 (m, 2H, pyrazoline-4'-H), 3.65–3.78 (m, 1H, C-17α-H), 5.39 and 5.44 (2d, 1H, pyrazoline-5'-H), 7.76 (s, 1H, C-4-H), 7.34–7.48 (m, 10H, Ar-H), 7.82 (s, 1H, C-1-H).

2-(3'-m-Tolyl-4',5'-dihydro-1'-phenylpyrazol-5'-yl)estradiol ***21***. This was obtained in 35% yield, mp: 178–180°C. Anal $C_{34}H_{38}N_2O_3$ (C, H, N).

2-[3'-(2-Furyl)-4',5'-dihydro-1'-phenylpyrazol-5'-yl)estradiol ***22***. This was obtained in 20% yield, mp: 159–160°C. Anal $C_{31}H_{34}N_2O_3$ (C, H, N).

2-(3'-Phenyl-4',5'-dihydro-1'-phenylpyrazol-5'-yl)estradiol 17β-acetate ***23***. This was obtained in 58% yield, mp: 185–186°C. Anal $C_{35}H_{38}N_2O_3$ (C, H, N).

2-(3'-m-Tolyl-4',5'-dihydro-1'-phenylpyrazol-5'-yl)estradiol 17β-acetate ***24***. This was obtained in 63% yield, mp: 195–198°C. Anal $C_{30}H_{40}N_2O_4$ (C, H, N). UV $\lambda_{max}^{methanol}$: 255 (4.5) and 305 (4.2) nm (log ε). ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H, 18-CH_3), 2.10 (s, 3H, C-17-$OCOCH_3$), 3.25–3.50 (d, dist, 2H, pyrazoline-4'-H), 3.85 (s, 3H, OCH_3), 4.52–4.80 (m, 1H, C-17α-H), 4.90–5.25 (m, 1H, pyrazoline-5'-H), 6.65 (s, 1H, C-4-H), 6.70–7.35 (m, 10H, 9Ar-H + C-1 -H).

2-[3'-(2-Furyl)-4',5'-dihydro-1'-phenylpyrazol-5'-yl]estradiol 17β-acetate ***25***. This was obtained in 25% yield, mp: 175–177°C. Anal $C_{33}H_{36}N_2O_4$ (C, H, N). UV $\lambda_{max}^{methanol}$: 265 (3.8) and 311 (3.2) nm (log ε).

2'-Aryl or 2'-furylestradiolpyran-4'-one ***26, 27***
A solution of **14** or **15** (0.2 mmol) in methanolic NaOH (pH 10, 30 ml) was left at rt for 3 d. The mixture was acidified with 10% aqueous HCl and extracted with ethyl acetate. The extracts were washed twice with water, dried (anhydrous Na_2SO_4) and evaporated to dryness. The residue was crystallized from ethanol. IR for compounds **26–27**: ν 3450 (OH), 1680 (C=O, pyrone) and 1610 (C=C aromatic) cm^{-1}.

2'-Phenylestradioldihydropyran-4'-one ***26***. This was obtained in 60% yield, mp: 210–212°C. Anal $C_{27}H_{30}O_3$ (C, H). ^{1}H-NMR ($CDCl_3$): δ 0.81 (s, 3H, 18-CH_3), 3.50 (d, 2H, *J* = 3Hz, pyrone-3'-H), 3.50–3.80 (t, dist, 1H, C-17α-H), 5.30–5.55 (dd, 1H, pyrone-2'-H), 6.75 (s, 1H, C-4-H), 7.20–7.52 (m, 5H, Ar-H), 7.82 (s, 1H, C-1-H).

2'-Furylestradioldihydropyran-4'-one ***27***. This was obtained in 55% yield, mp: 220–222°C. Anal $C_{28}H_{32}O_4$ (C, H). ^{1}H-NMR ($CDCl_3$): δ 0.80 (s, 3H, 18-CH_3), 3.50–3.85 (m, 4H, C-17α-H + OCH_3), 3.90–4.30 (d, 2H, *J* = 3 Hz, pyrone-3'-H), 5.20–5.35 (dd, 1H, pyrone-2'-H), 6.63 (s, 1H, C-4-H), 6.70–7.40 (m, 4H, Ar-H), 7.70 (s, 1H, C-1-H).

Binding affinity to the estrogen receptor

The synthesized compounds **1–13** and **26** were tested for their ability to compete with tritiated ([^{3}H]E_2) for the estrogen receptor from rat uterine homogenates using the centrifugal assay method [19].

Preparation of the uterine nuclear suspension [20]
Uteri (0.5 g) were isolated from the rats and were chilled in ice immediately upon removal. The fat was trimmed off and the uteri were washed with cold TE buffer (Tris–aminomethylidene trimethanol (10 mM), EDTA (1.5 mM); pH 7.4) frozen in liquid nitrogen, minced and stored at –70°C. The frozen uterine tissue was then homogenized in TE buffer (1 ml/100 mg uterine tissue) and the homogenate was centrifuged at 3000 *g* for 10 min. The obtained pellet was suspended in ice-cold TE buffer (2 ml), rehomogenized and centrifuged at 3000 *g* for 10 min. The supernatant was discarded and the pellet was suspended in TE buffer (5 ml) to obtain the nuclear suspension which was kept frozen until time of use.

Determination of the protein content
The protein content of the nuclear suspension was determined using the method described by Lowry *et al* [21].

Binding procedure
For the binding assay, 3 sets of assay tubes were prepared:
– For the determination of the specific and non-specific binding of [^{3}H]E_2, a nuclear suspension (100 µl) containing 90 µg protein was mixed with TE buffer (125 µl) containing [^{3}H]E_2 (25 µl).
– For the determination of non-specific binding, a nuclear suspension (100 µl, 90 µg protein) was mixed with TE buffer (75 µl) containing diethylstilbestrol (DES, 50 µl, 10^{-5} M in DMSO) and incubated for 15 min at 4°C prior to the addition of [^{3}H]E_2 (25 µl).
– For the determination of the binding affinity of the tested drugs, a nuclear suspension (100 µl, 90 µg protein) was added to a solution of each compound **1–13** and **26** (1 µl; 10^{-2} M) in TE buffer (124 µl) and incubated for 1 h at 4°C before the addition of [^{3}H]E_2 (25 µl). After incubation at 4°C for 18 h, the mixtures were treated with ethanol buffer (7% ethanol in TE buffer, 2 ml) and centrifuged at 3000 *g* for 10 min. The supernatant containing the free [^{3}H]E_2 was aspirated and the nuclear pellets containing bound [^{3}H]E_2 were suspended in toluene scintillation solution (0.055% PPO, 0.001% POPOP, 66.7% toluene and 33.3% Triton X-100), vortexed, and transferred quantitatively to scintillation counting vials and counted. The counts (cpm) obtained for samples with competitors tested relative to those without competitors were calculated to give the percentage competition (table I).

Uterotrophic activity [7, 9]

The uterotrophic activity of the synthesized compounds was evaluated by determining uterine weight gain in mature ovariectomized female albino rats (100–180 g) (obtained from the animal house of the Alexandria Faculty of Pharmacy). The compounds were administered sc once daily over a 4-d period in 0.1 ml of olive oil (0.09 µmol/rat/d). The rats were weighed 24 h after the final dose and vaginal smears were taken and examined under the microscope. The animals were then killed and the uteri carefully dissected, blotted, weighed, dried at 60°C for 24 h and weighed again. The gain in uterine weight calculated as mg uterine weight/100 g body weight and the percentage of dry/wet weight have been given in table II.

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