Bioorganic & Medicinal Chemistry Letters 18 (2008) 4102–4105

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and in vivo evaluation of 11-substituted estradiol derivatives as anti-implantation agents ☆

Indra Dwivedy [a], Atul Gupta [a,*], Arvinder Grover [a], Vandana Srivastava [a], Man Mohan Singh [b], Suprabhat Ray [a]

[a] Medicinal and Process Chemistry Division, Central Drug Research Institute, PO Box 173, Lucknow 226001, India
[b] Endocrinology Division, Central Drug Research Institute, PO Box 173, Lucknow 226001, India

ARTICLE INFO

Article history:
Received 8 March 2008
Revised 27 April 2008
Accepted 23 May 2008
Available online 29 May 2008

Keywords:
Estrogen receptor
Estrogen agonist
Estrogen antagonists
Antifertility
Anti-implantation
Steroids

ABSTRACT

Synthesis of 11-substituted estradiol derivatives (**12**–**17**) has been carried out by the Grignard reaction with alkyl, allyl, and benzyl halides on 17β-hydroxy-3-methoxy-11-oxo-estra-1,3,5(10),8(9)-tetraene (**10**). The novel compounds (**10** and **12**–**17**) were evaluated for their preliminary post-coital contraceptive (anti-implantation) activity in Sprague–Dawley rats. The tested compounds were administered orally and showed significant anti-implantation activity. Compound **13** is the most potent compound in the series which showed 100% contraceptive efficacy at 1.25 mg kg^{-1}.

Estrogens and progesterone are the hormones which are involved in the development of secondary sexual characteristics in female body. In reproductive cycle, the release of gonadotrophin-releasing hormone (GnRH) from pituitary triggered by low level of estrogen mainly estradiol (**1**) and progesterone (**2**) effects ovulation of a mature ovum from ovary (Fig. 1).[1] After ovulation, there is an increase in the level of estradiol (**1**) followed by that of progesterone (**2**), which stops further ovulation through a negative feedback mechanism. A critical estrogen–progesterone balance is necessary for implantation of the blastocyst and its subsequent development. Any alteration in this balance may lead to the termination of pregnancy. Steroidal pills which contain a combination of estrogen and progestagen create a pseudo-pregnancy situation by preventing ovulation.[2] However, such pills have some serious side effects such as nausea, pigmentation, breast tenderness and weight gain, due to creation of a state of pseudo-pregnancy.[3]

An alternate approach to the contraception is through the use of antiestrogens. In reproductive cycle, the level of estrogen rises two times. The first rise comes after the ovulation as mentioned before.[1] The second rise comes at the time of implantation of blastocyst. The suppression of this estrogen level causes implantation failure. Since this phenomenon happens at the peripheral end of the reproductive cycle, it does not create any disturbance in the cycle and is therefore devoid of pregnancy associated side effects (Fig. 2).

Several modifications have been made in the estradiol (**1**) and progesterone (**2**) with a view to get an ideal fertility regulating agent (contraceptive) with balanced agonistic and antagonistic activities.[4,5] Amongst various modifications, substitution at position 11 of an estrane and progestin led to the development of two important compounds, **3** and **4** (Fig. 1).

Our research program aimed for development of estrogen receptor modulators for fertility regulation and treatment of other estrogen dependent disorders, led to the development of first and only non-steroidal oral contraceptive, Ormeloxifene (centchroman, **5**).[6] Ormeloxifene (**5**), is a racemate mixture and marketed under the trade name of Saheli. It is a weekly pill and acts as an anti-implantation agent based on its antiestrogenic property.[7] The basic and significant difference between non-steroidal anti-implantation agents and steroidal pills (combination of estrogen and progestagen) is in their mechanism of action.

In our continuous efforts, we have designed and synthesized 11-substituted estradiol derivatives (**12**–**17**) as novel anti-implantation agents. The synthesized compounds were evaluated for their preliminary anti-implantation activity in Sprague–Dawley rat model.[8] The targeted compounds were expected to be orally active as desired from an ideal contraceptive (a fertility regulating agent).

☆ CDRI Communication No. 5322.

* Corresponding author at present address: University du Quebec à Trois-Rivieres, C.P. 500, Trois-Rivieres, Que., Canada G9A 5H7. Tel.: +91 522 2629558.
E-mail addresses: atisky2001@yahoo.co.in, atul_gupta04@yahoo.co.in (A. Gupta).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.093

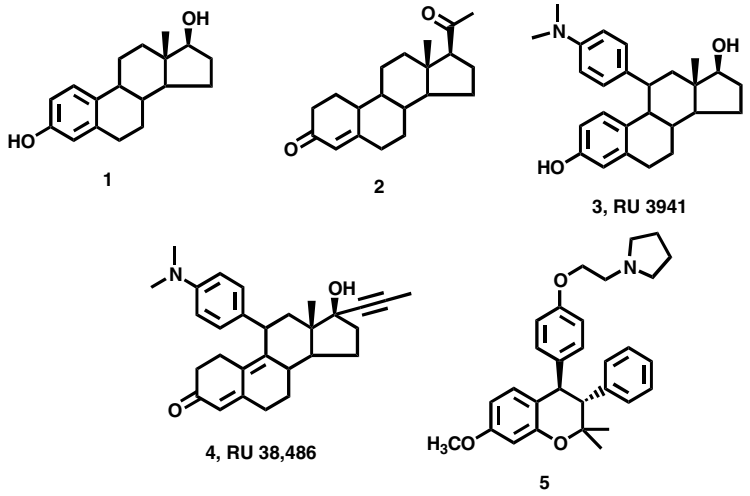


Figure 1. Some steroidal and non-steroidal fertility regulating agents.

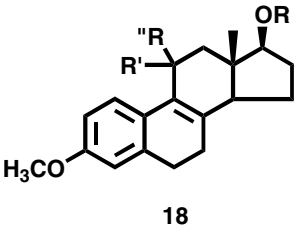
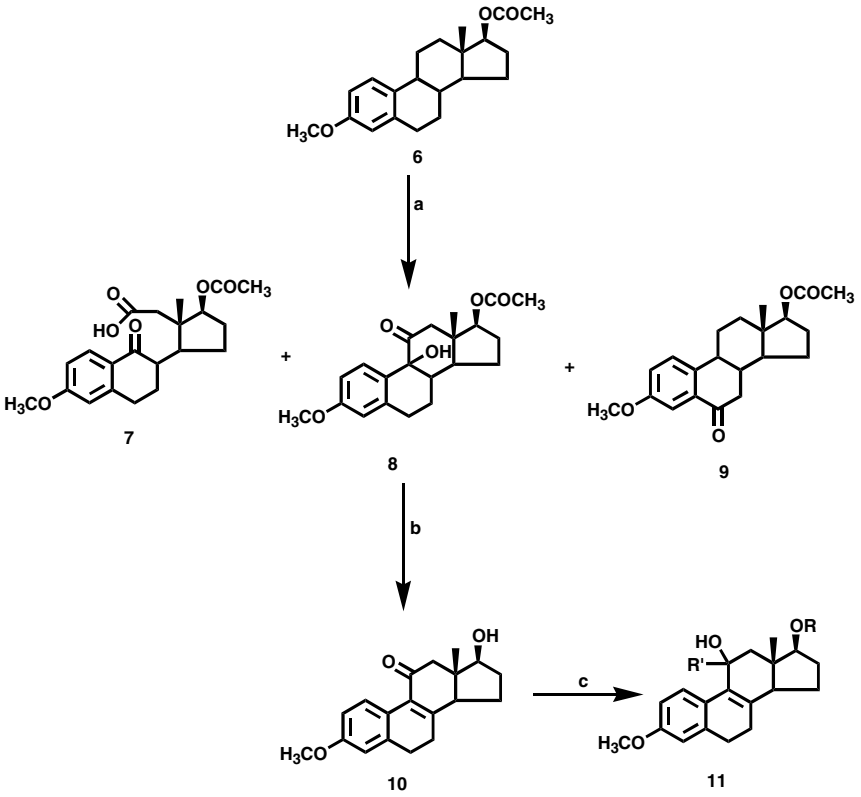


Figure 2. Designed prototype.

The synthesis of targeted compounds was started from 17β-hydroxy-3-methoxy-1,3,5(10)-estratriene 17-acetate (**6**) (Scheme 1). The CrO_3 oxidation of **6** using reported procedure, gave 9α,17β-dihydroxy-3-methoxy-11-oxo-estra-1,3,5(10)-triene 17-acetate (**8**) in 50 % yield along with 17β-hydroxy-3-methoxy-9-oxo-9,11-secoestra-1,3,5(10)-triene-11- oic acid 17-acetate (**7**) and 17β-hydroxy-3-methoxy-6-oxo-estra-1,3,5(10)-triene 17-acetate (**9**).[9] Compound **8**, on treatment with perchloric acid in methanol at reflux temperature for 1 h, yielded 17β-hydroxy-3-methoxy-11-oxo-estra-1,3,5(10),8(9)-tetraene (**10**) in 76 % yield. Grignard reaction

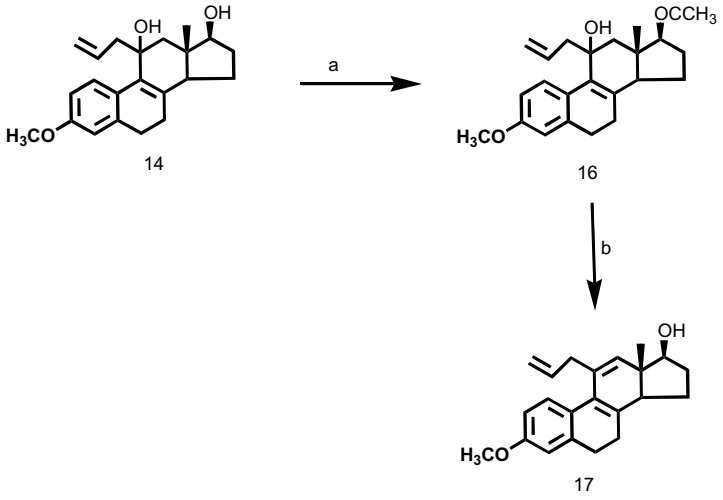


Scheme 1. Reagents and conditions: (a) CrO_3, glacial acetic acid, room temperature (RT); (b) Perchloric acid, MeOH, reflux; (c) Methyl/ethyl/allyl/benzyl halide, Mg, dry Et_2O–THF, room temperature (RT).

was made on compound **10** with alkyl, allyl, and benzyl magnesium halides in dry Et_2O/THF mixture at room temperature (RT), and gave the desired product **11** in 60–76% yields.[10]

Furthermore, to evaluate the significance of hydroxyl group at position 11 of the steroidal nucleus in these compounds, compound **14** was converted to 11-allyl-17β-hydroxy-3-methoxy-estra-1,3,5(10),8(9),11-pentaene (**17**) (Scheme 2). For the synthesis of compound **17**, **14** was transformed to the corresponding mono acetate (**16, 17β-acetate**) with acetic anhydride in pyridine which was dehydrated with *p*-toluenesulfonic acid in benzene at reflux to give **17** in 35% yield. The synthesized compounds were characterized by the use of IR, NMR, mass spectroscopy, and their elemental analysis.[9]

Biological activity of the compounds (**10** and **12**–**17**) is given in the Table 1. The novel compounds showed potent anti-implantation activity when administered orally in Sprague–Dawley rat model. The structure–activity relationship (SAR) of these compounds showed that moderately sized and flexible substituents like ethyl and allyl groups find better adjustment in the ligand-binding domain (LBD) of the estrogen receptor. This is in agreement with the reported fact in the literature that small to moderate sized substituents at the 11-position of estrogens are well tolerated by the estrogen receptor.[11] The ethyl-substituted derivative, **13**, is the most potent compound in the series which showed 100% contraceptive efficacy at 1.25 mg kg^{-1} in rat model. However, substitution with benzyl group brought a drop in the activity possibly due to the presence of a bulky rigid ring system (benzene ring) as compared to allyl group. Further, the low order of anti-implantation activity of compounds **16** and **17** showed the significance of the hydroxyl group at 11 and 17 positions of the steroidal



Scheme 2. Reagents and conditions: (a) acetic anhydride, pyridine, room temperature (RT); (b) *p*-Toluenesulfonic acid, dry benzene, reflux.

Table 1
Anti-implantation activity of compounds

Compound	R	R′	R″	Dose (mg kg^{-1}, body weight/ No. of animals)[a,c]	Route	Schedule (day of treatment)[b]	No. of implantation sites	Anti-implantation activity (%)
Control				—	Oral	1–5	10/10	All rats pregnant
Centchroman				10.0	Oral	1–5	0/10	100
Centchroman				1.00	Oral	1–5	0/10	100
10	H	C=O at C11	—	10.0	Oral	1–5	0/10	100
10	H	C=O at C11	—	5.0	Oral	1–5	0/10	100
10	H	C=O at C11	—	2.0	Oral	1–5	10/10	Inactive
12	H	CH_3	OH	10.0	Oral	1–5	0/10	100
12	H	CH_3	OH	5.0	Oral	1–5	0/10	100
12	H	CH_3	OH	2.0	Oral	1–5	0/10	100
13	H	CH_2CH_3	OH	10.0	Oral	1–5	0/10	100
13	H	CH_2CH_3	OH	5.0	Oral	1–5	0/10	100
13	H	CH_2CH_3	OH	2.5	Oral	1–5	0/10	100
13	H	CH_2CH_3	OH	1.25	Oral	1–5	0/10	100
13	H	CH_2CH_3	OH	1.00	Oral	1–5	5/10	50
14	H	CH_2=$CHCH_2$	OH	10.0	Oral	1–5	0/10	100
14	H	CH_2=$CHCH_2$	OH	5.0	Oral	1–5	0/10	100
14	H	CH_2=$CHCH_2$	OH	2.0	Oral	1–5	0/10	100
14	H	CH_2=$CHCH_2$	OH	1.5	Oral	1–5	4/10	60
14	H	CH_2=$CHCH_2$	OH	1.0	Oral	1–5	4/10	60
15	H	$CH_2C_6H_5$	OH	10.0	Oral	1–5	0/10	100
16	CH_3CO	CH_2=$CHCH_2$	OH	10.0	Oral	1–5	0/10	100
17	H	CH_2=$CHCH_2$	Double bond (C11–C12)	10.0	Oral	1–5	0/10	100

Compounds described in the table are based on structure **18** (Fig. 2).
[a] Five animals were used for each dose test.
[b] For schedule of treatment, day **1** was counted from day **1** of the pregnancy.
[c] Control rats did not receive any drug, had a mean of 10 implantation sites (visibly thickened uteri).

nucleus which seemed to be essential for the biological activity of these novel compounds.

In conclusions, the newly synthesized 11-substituted estradiol derivatives (**12**–**17**) showed significant oral anti-implantation activity in Sprague–Dawley rat model. As desired, the target compounds are orally active. Minimum effective dose of the tested compounds was determined. The potent biological activity (anti-implantation activity) of these novel compounds showed successful drug design. Further, investigation of these estradiol derivatives would be helpful in designing of the compounds for fertility regulation as anti-implantation agents as well as estrogen receptor modulators.

Acknowledgments

Atul Gupta thanks CSIR (India) for Senior Research Fellowship. The authors thank Miss Mohini Chhabra for efficient technical assistance and the Ministry of Health and Family Welfare, Government of India, for financial support.

References and notes

1. (a) Ray, S.; Dwivedy, I. *Adv. Drug Res.* **1997**, *29*, 171; (b) Lednicer, D. *The Chemical Control of Fertility*; Marcel Dekker: New Yark, 1969.
2. (a) Goldzieher, J. W. *Perspect. Biol. Med.* **1993**, *36*, 363; (b) Perone, N. *Perspect. Biol. Med.* **1993**, *36*, 347.
3. (a) Vojta, M. *Ceskoslovenska Gynekol.* **1971**, *36*, 273; (b) Plotz, E. J. *Geburtshilfe Frauenheilked* **1970**, *30*, 193.
4. Claussner, A.; Nedelec, L.; Nique, F.; Philibert, D.; Teutsch, G.; Vande Velde, P. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 609.
5. Ray, S.; Sharma, I. *Phramazie* **1987**, *42*, 656.
6. (a) Salman, M.; Ray, S.; Agarwal, A. K.; Durani, S.; Setty, B. S.; Komboj, V. P.; Anand, N. *J. Med. Chem.* **1983**, *26*, 592; (b) Singh, M. M. *Med. Res. Rev.* **2001**, *21*, 302.
7. Ray, S.; Singh, M. M.; Agarwal, A. K.; Komboj, V. P. *Contraception* **1987**, *35*, 283.
8. Ghosh, R.; Kamboj, V. P.; Singh, M. M. *Contraception* **2001**, *64*, 261.
9. (a) Cambie, R. C.; Manning, T. D. R. *J. Chem. Soc.* **1968**, *21*, 2603; (b) Dygos, J. H.; Chin, L. J. *J. Org. Chem.* **1975**, *40*, 685.
10. *Experimental:*
*Synthesis of 9α, 17β-dihydroxy-3-methoxy-11-oxo-estra-1,3,5(10)-triene 17-acetate (***8***):* A solution of chromium trioxide (4.25 g, 0.04 mol) in distilled water (2 ml) and glacial acetic acid (30 ml, $KMnO_4$ treated) was added dropwise to a stirred solution of 3,17β-dihydroxy-estra-1,3,5(10)-triene 17-acetate,3-methyl ether (**6**) (5.0 g, 0.02 mol) in glacial acetic acid (30 ml) kept at 5–10 °C. After complete addition, stirring was continued for 15 h and methanol (2 ml) was added to decompose excessive CrO_3. The reaction mixture was diluted with water and extracted with ethyl acetate, dried over anhydrous sodium sulfate, and concentrated to oily residue. The crude residue was purified over a column of silica gel and eluted with ethyl acetate–hexane (3:47) to get pure compound **8**.
Yield: 50%, mp 115–117 °C. FABMS: (M+) 358, (M+1) 359, (M+2) 360; IR (cm^{-1}): 3465, 1732, 1611, 1244; ^1H NMR (δ, $CDCl_3$): 0.82 (s, 3H, CH_3), 1.57 (s, 2H, CH_2), 1.57–1.83 (m, 2H, CH_2), 2.04 (s, 3H, CH_3), 2.08–2.54 (m, 6H, CH and CH_2), 2.79 (br s, 2H, CH_2), 4.41 (br s, 1H, OH), 3.77 (s, 3H, OCH_3), 4.71 (t, 1H, CH), 6.70 (br s, 3H, ArH); Anal. Calcd for $C_{21}H_{26}O_5$; C, 70.37; H, 7.31. Found: C, 70.50; H, 7.48.
*Synthesis of 3-methoxy-17β-hydroxy-11-oxo-estra-1,3,5(10),8(9)-tetraene (***10***):* A solution of compound **8** (4.0 g, 0.011 mol) in menthol (60 ml) and perchloric acid (2 ml) was refluxed for 1 h. Methanol was removed under vacuum, residue was dissolved in ethyl acetate and extracted with water. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated to oily residue which gave compound **10** (2.5 g, 76%) after crystallization with benzene–hexane.
Yield: 76%; mp 140–142 °C; IR (cm^{-1}): 1650 and 1600 (aromatic); mass: (M^+) 298; ^1H NMR (δ, $CDCl_3$): 1.10 (s, 3H, CH_3), 1.86–2.05 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.15 (t, 1H, CH), 6.40–6.60 (m, 2H, ArH), 7.80–8.00 (d, 1H, J = 8.00 Hz, ArH); ^{13}C NMR (δ, $CDCl_3$): 197, 159, 158, 137, 129, 128, 124, 113, 111, 80, 55, 49, 47, 47, 33, 30, 29, 28, 20; Anal. Calcd for $C_{19}H_{22}O_3$; C, 76.49; H, 7.43. Found: C, 76.30; H, 7.40.
*Typical procedures for the synthesis of 11-allyl-11,17β-dihydroxy-3-methoxy-estra-1,3,5(10),8(9)-tetraene (***14***):* Compound **10** (1.0 g, 0.003 mol) in dry tetrahydrofuran (THF) was added dropwise to a stirred mixture of allyl magnesium bromide (0.017 mol) in dry ether (Et_2O) under inert atmosphere. The reaction mixture was further stirred for2 h. On completion of the reaction, reaction mixture was decomposed with water, extracted with ether, dried over anhydrous sodium sulfate, and concentrated at room temperature. The resulting oil (0.8 g, 74%) was filtered through a column of neutral alumina. The eluted fractions were concentrated under vacuum to give pure compound **14**.
Yield: 74 %; mp. Oil; IR (cm^{-1}): 1620 and 1500 (aromatic); mass: (M^+) 340, (M^+-H_2O) 322, (M^+–allyl) 299, (M^+–allyl+H_2O) 281; ^1H NMR (δ, $CDCl_3$): 1.15 and 1.17 (2s, 3H, CH_3), 2.10 (m, 2H, CH_2—CH=CH_2), 2.80 (br s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.21 (t, 1H, CH), 4.80–5.10 (m, 3H, CH=CH_2), 6.40–6.60 (m, 2H, ArH), 7.80–8.00 (d, 1H, J = 8.00 Hz, CH); Anal. Calcd for $C_{22}H_{28}O_3$; C, 77.61; H, 8.28. Found: C, 77.41; H, 8.18.
*Synthesis of 11-allyl-11,17β-dihydroxy-3-methoxy-estra-1,3,5(10),8(9)-tetraene 17-acetate (***16***):* A mixture of compound **14** (0.1 g, 0.0003 mol) in dry pyridine (2 ml) and acetic anhydride (0.2 ml) was allowed to stand for 12 h at room temperature. Water was added to the reaction mixture and extracted with ether. The organic layer was separated and dried over anhydrous sodium sulfate and concentrated to give compound **16** (0.75 g) as oil.
Yield: 64%; mp. Oil; IR (cm^{-1}): 3400, 1710, 1600 (aromatic); Mass: (M^+) 382, (M^+-H_2O) 364, (M^+-COCH_3) 340; ^1H NMR (δ, *CDCl*$_3$): 1.00 (1s, 3H, CH_3), 1.80 (1s, 3H, CH_3), 2.12 (m, 2H, CH_2—CH=CH_2), 2.81 (b rs, 2H, CH_2), 3.60 (s, 3H, OCH_3), 4.21 (t, 1H, CH), 4.50–5.00 (m, 3H, CH=CH_2), 6.50–6.70 (m, 2H, ArH), 7.90 (d, 1H, J = 8.00 Hz, CH); Anal. Calcd for $C_{24}H_{30}O_4$; C, 75.36; H, 7.91. Found: C, 75.50; H, 7.80.
*Synthesis of 11-allyl-17β-hydroxy-3-methoxy-estra-1,3,5(10),8(9),11-pentaene (***17***):* A mixture of compound **16** (0.32 g, 0.001 mol) and *p*-toluenesulfonic acid (0.05 g) in dry benzene was refluxed in a Dean–Stark apparatus for 20 min. The benzene was evaporated off; water was added to the reaction mixture and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to give a crude oily residue. The crude residue was purified on a column of silica gel which gave pure compound **17** as oil.
Yield: 35%; mp. Oil; IR (cm^{-1}): 1680, 1580 (aromatic); mass: (M^+) 322; ^1H NMR (δ, *CDCl*$_3$): 1.01 (1s, 3H, CH_3), 2.65 (m, 2H, CH_2—CH=CH_2), 2.80 (br s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 4.23 (t, 1H, CH), 4.80–5.10 (m, 3H, CH=CH_2), 5.40 (s, 1H, CH), 7.00–7.60 (m, 3H, ArH); Anal. Calcd For $C_{22}H_{26}O_2$; C, 81.94; H, 8.10. Found: C, 81.69; H, 8.00.
Anti-implantation screening:[8] Anti-implantation activity of the compounds was studied in sperm positive adult (180–220 g) Sprague–Dawley female albino rats mated to coeval males of proven fertility. The compounds were administered orally as a suspension in gum acacia to colony bread adult mated female rats on days 1–5 post-coitum using five to seven animals in each group. The animals were examined by laprotomy on day 10 of pregnancy for the number and status of implantations and corpora lutea. The results were scored as positive only if implantations were totally absent in both the uterine horns of each animal.
11. Bindal, R. D.; Carlson, K. E.; Reiner, G. C. A.; Katzenellenbogen, J. A. *J. Steroid. Biochem.* **1987**, *28*, 361.