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New-*D*-homoandrost-4,6-diene derivatives as potent progesterone receptor antagonist

Marisa Cabeza[a,*], Mario García-Lorenzana[b], Montserrat Garcés[a], Ivonne Heuze[a], Nayeli Teran[c], Eugene Bratoeff[c]

[a] Department of Biological Systems and Animal Production Metropolitan University-Xochimilco, Mexico D. F., Mexico
[b] Department of Biology of Reproduction, Metropolitan University-Iztapalapa, Mexico D. F., Mexico
[c] Department of Pharmacy, Faculty of Chemistry, National University of Mexico City, Mexico D. F., Mexico

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ABSTRACT

The aim of this study was to synthesize three different *D*-homoandrostadiene derivatives (**2**–**4**) and study their biological activity. We carried out *in vivo* and *in vitro* experiments using female cycling mice, which were synchronized for estrus with luteinizing hormone-releasing hormone (LHRH) and injected with the steroidal compounds. It was also determined the binding of these compounds to the progesterone receptors (PR). Since these steroids have a new *D*-homoandrostandienone skeleton in their molecular structure, it was of interest also to study their binding to the androgen receptors (AR).

After LHRH treatment, the mice of the control group showed the presence of 14 ± 4 corpus lutea in the ovary whereas the animals treated with steroids **2**–**4**, with RBAs of 100%, exhibited 11 ± 7, 12 ± 2, and 10 ± 4 respectively. As a result of this study, it is evident that these steroids did not inhibit the ovulation in these animals.

The uterus of the control group, showed the typical progestational activity with an enlarged endometrial thickness with a secretory activity. However, the endometrium of the mice treated with steroids **2**–**4** did not show an enlargement of the endometrium and no secretory activity could be detected. This fact indicates that compounds **2**–**4** had antagonistic activity in this tissue.

The overall data show that steroids **2**–**4** are antagonists of the PR. However, they do not bind to the AR. These results also demonstrate that **2**–**4** have an antiprogestational activity *in vivo*, but do not decrease the number of corpus lutea in the ovary of mice treated with LHRH.

1. Introduction

Blocking progesterone receptor (PR) function by using PR antagonists should allow the modulation of various reproductive processes. On this basis antiprogestins were developed which disrupt the normal progesterone-induced signal transduction pathway by competitive binding to PR. Therefore, antiprogestins have considerable potential as therapeutic drugs for numerous gynecological, obstetrical and oncological afflictions as well as contraceptives since these compounds can block ovulation [1] and prevent implantation [2].

Antiprogestins, such as mifepristone **1** (Fig. 1), have potential use for both regular and emergency contraception and for the treatment of hormone-related pathological conditions such as breast cancer, endometriosis and uterine leiomyomas. Mifepristone **1** (RU 486) was the first steroid of this class to show high affinity for binding to the progesterone receptor (PR) [3,4] and was recommended for the treatment of endometriosis [5]. However, mifepristone induces the luteolysis in pregnant mice [6] and inhibits gonadotropin activity in women [7]. These are not desirable effects for the treatment of sterility produced by endometriosis since the ovulation is inhibited. Mifepristone also produces side effects that include hot flushes and transient increases in liver transaminases [5].

Previously, we synthesized several progesterone derivatives containing a phenylacetyloxy substituent at C-17 which bind selectively to the PR and showed an antiprogestin activity [8]. These steroids inhibited the ovulation and interrupted the endometrial maturation of estrous mice. Since these compounds inhibited the ovulation it was of interest to synthesize similar *D*-homoandrostadiene derivatives having a phenyl substituent at C-16, methyl and phenylacetoxy functions at C-17 (compound **2**) or a fluorophenyl acetoxy group at C-17 (compounds **3** and **4**) and

* Corresponding author at: Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso No. 1100, Col Villa Quietud, México, D.F., C.P. 04960, Mexico. Tel.: +52 55 5483 72 60; fax: +52 55 5483 72 60.
E-mail address: marisa@correo.xoc.uam.mx (M. Cabeza).

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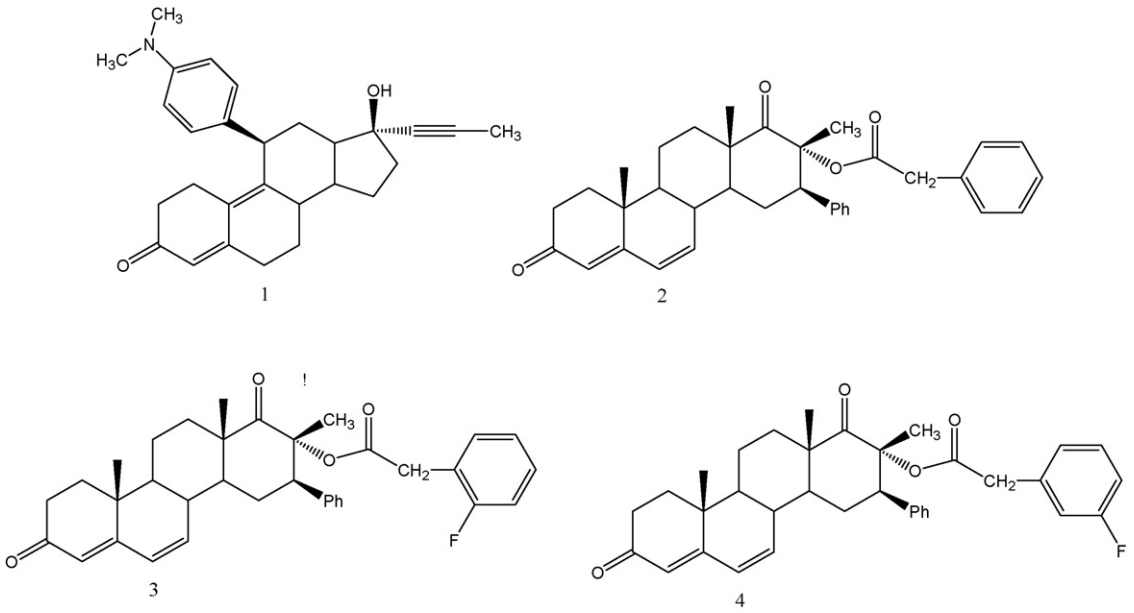


Fig. 1. Structures of mifepristone and the novel steroids **2**–**4**.

determine the antiprogestational effect in the endometrium without blocking ovulation or to produce degenerative changes in the ovary.

The aim of this research was to synthesize three different *D*-homoandrostadiene derivatives (Fig. 2): 17β-Methyl-16β-phenyl-17α-phenylacetoxy-*D*-homoandrost-4,6-diene-3,17a-dione (**2**), 17β-methyl-16-β-phenyl-17α-(2-fluorophenyl)acetoxy-*D*-homoandrost-4,6-diene-3,17a-dione (**3**) and 17β-methy-16β-phenyl-17α-(3-fluorophenyl)acetoxy-*D*-homoandrost-4,6-diene-17a-dione (**4**) and to study their activities as potent progesterone receptor antagonists. It was also of interest to determine the binding of these steroids to the androgen receptor (AR). Furthermore we also examined the effect of these compounds in the ovulation, as well as their function as interrupters of endometrial maturation.

2. Materials and methods

2.1. Chemical and radioactive material

Solvents were laboratory grade or better. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were taken on Varian gemini 200 and VRX-300, respectively. Chemical shifts are given in ppm relative to that of Me_4Si ($\delta = 0$) in $CDCl_3$ (the abbreviations of signal patterns are as follows: s, singlet; d, doublet, t, triplet, m, multiplet, q, quartet). Mass spectra were obtained with an HP5985-B spectrometer. IR spectra were recorded on a PerkinElmer 200 s spectrometer.

The radioligands: promegestone (17α-methyl-^{3}H) [^{3}H] R5020 (synthetic progestin with high affinity for the PR [9]) specific activity of 87 Ci/mmol; mibolerone (17α-methyl-^{3}H) [^{3}H] MIB (synthetic androgen with high affinity by the AR) specific activity of 70–87 Ci/mmol, were provided by PerkinElmer Life Sciences, Inc. (Boston, MA). Radio inert mibolerone and R5020, were supplied by Steraloids (Wilton, NH, USA) and PerkinElmer Life Sciences, Inc. (Boston, MA), respectively. DL-Dithiothreitol and protease inhibitors were purchased from Sigma–Aldrich (St. Louis, MO, USA). Activated charcoal (acid washed with hydrochloric acid) and Dextran (Mr-70,000) were supplied by Sigma–Aldrich (St. Louis, MO).

2.2. Synthesis of steroidal derivatives

The synthesis of the new steroids **2**–**4** is briefly described below. The preparation of the intermediates **6**–**11** is given in Ref. [10].

A mixture of the corresponding phenyl acetic acid (1.79 mmol), p-toluenesulfonic acid (0.001 g, 0.0052 mmol) and trifluoroacetic anhydride (0.19 g, 0.81 mmol) was stirred for 2 h at room temperature. Steroid **11** (0.2 g, 0.49 mmol) was added; the reaction mixture was stirred for an additional 2 h at room temperature (nitrogen atmosphere). It was neutralized with an aqueous sodium bicarbonate solution to pH 7 and diluted with chloroform (10 mL). The organic phase was separated and dried over anhydrous sodium sulfate; the solvent was eliminated in vacuum. The crude product was purified by silica gel column chromatography and recrystallized from methanol.

2.2.1. 17β-Methyl-16β-phenyl-17α-phenylacetoxy-D-homoandrost-4,6-diene-3,17a-dione (**2**)

Yield 0.12 g, 0.19 mmol (39%) of pure product **2**, m.p. 186–189 °C. UV (nm): 283 (ε = 22,900). IR (KBr) cm^{-1}: 1735, 1720, 1704, 1667, 1620, 870, 761, 700. ^{1}H NMR ($CDCl_3$) δ: 1.10 (3H, s, H-18), 1.17 (3H, s, H-19), 1.26 (3H, s, methyl at C-17), 5.4 (2H, s, $COOCH_2$-Ph), 5.68 (1H, s, H-4), 6.13 (1H, d, J = 8.8 Hz, H-6), 6.26 (1H, q, J_1 = 8.8 Hz, J_2 = 2 Hz, H-7), 7.23 (5H, m, phenyl at C-16), 7.35 (5H, m, phenyl of ester). ^{13}C NMR ($CDCl_3$) δ: 16.2 (C-18), 17.6 (C-19), 22.9 (methyl at C-17), 123.8 (C-4), 128.2 (C-6), 139.2 (C-7), 162.2 (C-5), 170.7 (ester carbonyl), 199.4 (C-3), 211.6 (C-17a). FAB-MS (m/z) calcd for $C_{35}H_{38}O_4$ 523.5736 (M+H), found 523.5654.

2.2.2. 17β-Methyl-16β-phenyl-17α-(2-fluorophenyl) acetoxy-D-homoandrost-4,6-diene-3,17a-dione (**3**)

Yield 0.1 g, 0.19 mmol (37.4%) of pure product 3 m.p. 192–194 °C UV (nm) 284 (ε = 22,700). IR (KBr) cm^{-1}: 1730, 1712, 1662, 1618, 877. ^{1}H NMR ($CDCl_3$) δ: 1.11 (3H, s, H-18), 1.15 (3H, s, H-19), 1.22 (3H, s, methyl at C-17), 5.4 (2H, $COOCH_2$-Ph), 5.67 (1H, s, H-4), 6.13 (1H, d, J = 8.8 Hz, H-6), 6.27 (1H, q, J_1 = 8.8 Hz, J_2 = 2 Hz, H-7), 7.04 (5H, m, phenyl at C-16), 7.26 (4H, m, phenyl of ester). ^{13}C NMR ($CDCl_3$) δ: 16.4 (C-18), 18.0 (C-19), 23.1 (methyl at C-17), 127.4 (C-4), 128.1 (C-6), 128.4 (C-para, aromatic, 1C), 129.1 (C-ortho,

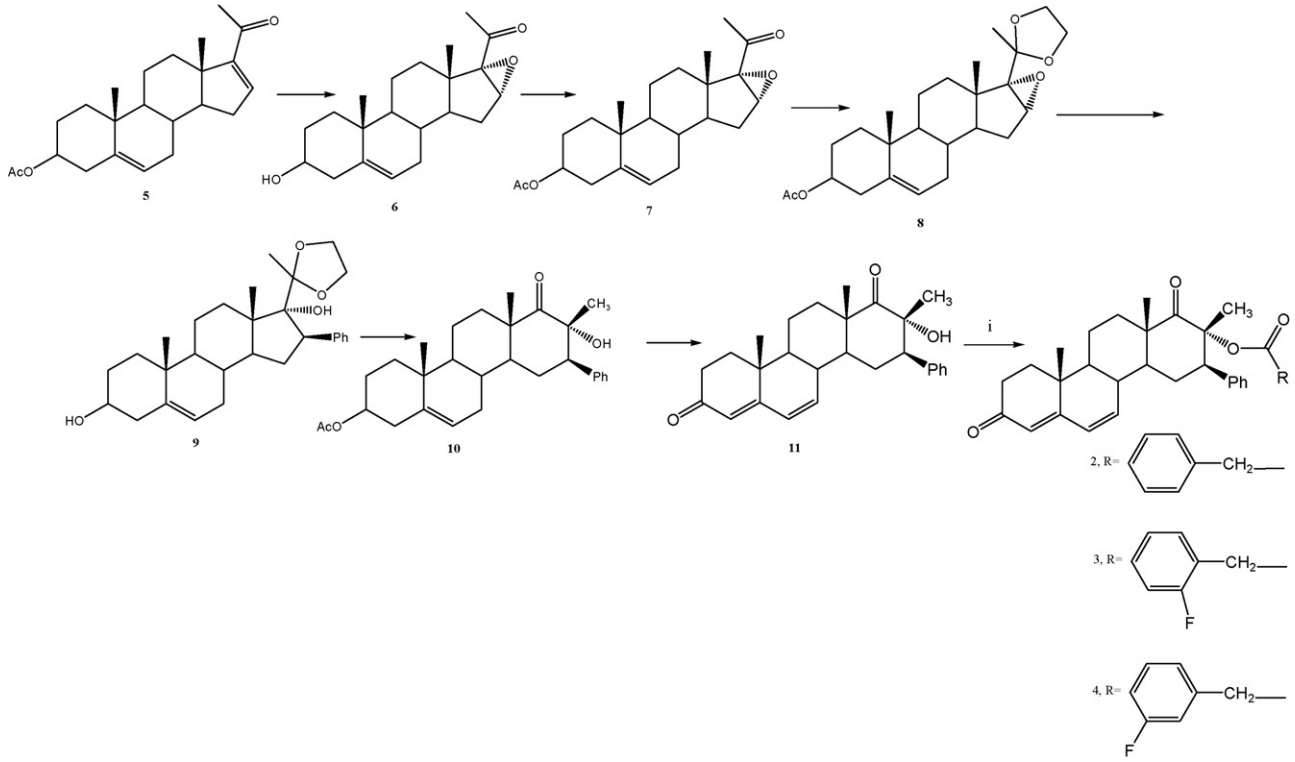


Fig. 2. Conditions and reagents: the preparation of compounds **5**–**11** is given in Ref. [10]. (i) $(CF_3CO)_2O$, PTSA, RCOOH, r.t., 3 h.

aromatic, 1C), 129.5 (C-meta aromatic, 2C), 130.2 (C-F aromatic, 1C), 138.5 (C-7), 162.9 (C-5), 170.2 (ester carbonyl), 199.9 (C-3), 211.8 (C-17a). FAB-MS (*m*/*z*) calcd for $C_{35}H_{37}FO_4$ 541.3412 (M+H), found 541.3465.

2.2.3. 17β-Methyl-16β-phenyl-17α-(3-fluorophenyl) acetoxy-D-homoandrost-4,6-diene-3,17a-dione (*4*)

Yield 0.1 g, 0.19 mmol (37.4%) of pure product **4** m.p. 178–180 °C UV (nm): 285 (ε = 22,500). IR (KBr) cm^{-1}: 1725, 1711, 1665, 1620. ^1H NMR ($CDCl_3$) δ: 1.11 (3H, s, H-18), 1.14 (3H, s, H-19), 1.23 (3H, s, methyl at C-17), 5.4 (2H, s, $COOCH_2$-Ph), 5.70 (1H, s, H-4), 6.15 (1H, d, J = 8.8 Hz, H-6), 6.25 (1H, q, J_1 = 8.8 Hz, J_2 = 2 Hz H-7), 6.91 (5H, m, phenyl at C-16), 7.37 (4H, m, phenyl of ester). ^{13}C NMR ($CDCL_3$) δ: 16.22 (C-18), 17.73 (C-19), 22.85 (methyl at C-17), 123.85 (C-4), 125.26 (C-6), 127.18 (C-para, aromatic, 1C), 128.12 (C-ortho, aromatic, 2 C), 128.75 (C-meta, aromatic, 1C), 130.11 (C-F, aromatic, 1C), 138.10 (C-7), 163.90 (C-5), 172.21 (ester carbonyl) 199.41 (C-3), 211.45 (C-17a). FAB-MS (*m*/*z*) calcd for $C_{35}H_{37}FO_4$ 541.3513 (M+H), found 541.3526.

2.3. Biological activity of the progesterone derivatives

The biological activity of **2**–**4** (Fig. 2) was determined in *in vivo* and *in vitro* experiments using CD1 female mice and adult New Zealand white female rabbits. The animals (mice 20–25 g and rabbits 4 kg) were obtained from the Metropolitan University-Xochimilco of Mexico. At the end of the *in vivo* experiment, the mice were sacrificed by cervical dislocation. In order to obtain the cytosol from the uteri of rabbits, the animals were sacrificed with CO_2. This protocol was approved by the Institutional Care and Use Committee of the Metropolitan University of Mexico. The uteri of the rabbits were removed, blotted, weighed in ice bath and soaked in cold TEMD (40 mM Tris–HCl, 3 mM EDTA and 20 mM sodium molybdate, dithiothreitol 0.5 mM, 10% glycerol at pH 7.4) and maintained in ice bath prior to their use.

2.4. Receptor binding assays

In this study we determined the binding of compounds: **2**–**4** to the progesterone receptors. Since these steroids have a new *D*-homoandrostandienone skeleton in their molecular structure, it was of interest also to determine their binding to the androgen receptors.

2.4.1. Androgen receptor (AR)

Rat prostates were homogenized with a tissue homogenizer (Teckmar, Cincinnati, OH), in one volume of buffer TEMD at pH 8 and protease inhibitors (2 mM PMSF, 10 μg/mL antipain, 5 mM leupeptin [11]) in ice bath with a tissue homogenizer. Homogenates were centrifuged at 140,000 × *g* for 60 min [12] in a SW 60 Ti rotor (Beckman Instruments, Palo Alto, CA).

The cytosolic fraction obtained from the supernatant liquid of the rat prostate homogenate described above, was stored at −70 °C. Prostatic cytosol proteins (4 mg of protein in 200 μL) were determined by the Bradford method [13].

For competitive studies, tubes containing 1 nM of [^3H] MIB plus a range of increasing concentrations (1×10^{-10} to 4×10^{-7} M) of cold MIB or steroids **2**–**4** in ethanol or chloroform, or in the absence of the competitor were prepared [14]. Incubates also contained 200 nM triamcinolone, in ethanol (Sigma), to prevent interaction of MIB with glucocorticoid receptors and progesterone receptors [14]; the solvent was completely eliminated.

Aliquots of 200 μL of prostate cytosol were added and incubated in the presence of 300 μL of TEMD buffer containing protease inhibitors (duplicate) for 18 h at 4 °C in the tubes as previously described [11]. After incubation 0.27 mL of saturated ammonium sulfate in TEMD buffer (35%) was added [15]. The mixture was further incubated for 1 h with occasional shaking for the precipitation of the [^3H] MIB-complex. The precipitate was collected by centrifugation at 10,000 × *g* for 10 min and the pellet was redissolved in 0.5 mL of TEMD and mixed with 0.5 mL of 0.1% dextran-coated 1%

charcoal in TEMD buffer. The mixture was incubated for 10 min at 4 °C. To prepare the dextran-coated charcoal mixture, the dextran was agitated for 30 min before adding the charcoal to the mixture. The mixture was agitated for two more hours. The tubes were vortexed and immediately centrifuged at 800 × *g* for 10 min to pellet the charcoal; aliquots (600 μL) were taken and submitted for radioactive counting using Ultima Gold (Packard) as counting solution. The radioactivity was determined in a scintillation counter (Packard tri-carb 2100 TR). The IC_{50} of each compound was calculated according to the plots of concentration versus percentage of binding.

2.4.2. Progesterone receptor (PR)

Uteri were isolated from mature rabbits treated for 7 days with 10 μg/animal/estradiol valerate. The uteri from the rabbits were minced and homogenized in equal volume of TEMD buffer plus protease inhibitors (2 mM PMSF, 10 μg/mL antipain, 5 mM leupeptin [9]; 40 mM Tris–HCl, 3 mM EDTA and 20 mM sodium molybdate, dithiothreitol 0.5 mM, 10% glycerol at pH 7.4) [8]. The homogenate was centrifuged at 140,000 × *g* for 1 h at 0 °C in a SW 60 Ti rotor (Beckman Instruments, Palo Alto, CA).

The cytosolic fraction obtained from the supernatant liquid of the rabbit uteri homogenate described above, was stored at −70 °C. Uteri cytosol proteins (4 mg of protein in 200 μL) were determined by the Bradford method [13].

For competitive studies, tubes containing 0.4 nM of [^{3}H] R5020 [15]; plus a range of increasing concentrations (1×10^{-10} to 4×10^{-6} M) of cold R5020, Mifepristone (RU486) or steroids **2**–**4** (1×10^{-10} to 4×10^{-6} M) in ethanol or chloroform, or in the absence of the competitor were prepared. In all tubes the solvent was eliminated in vacuum. We determined also the binding of the antiprogestin Mifepristone (RU486) to the PR using different concentrations of this steroid (1×10^{-10} to 4×10^{-6} M) and 0.4 nM of [^{3}H] R5020 for the competitive binding.

Aliquots of 200 μL of uteri cytosol (4 mg of protein) were added and incubated in the presence of 300 μL of TEMD buffer containing protease inhibitors in the tubes (duplicate) for 18 h at 4 °C as previously described [11]. After incubation 0.21 mL saturated ammonium sulfate in TEMD buffer (30%) was added [8,16,17]. The mixture was further incubated for 1 h with occasional shaking for precipitation of the [^{3}H] R5020-complex. The precipitate was collected by centrifugation at 10,000 × *g* for 10 min and the pellet was redissolved in 0.5 mL of TEMD and mixed with 0.5 of 0.1% dextran-coated 1% charcoal in TEMD buffer. The mixture was incubated for an additional 10 min at 4 °C. The tubes were vortexed and immediately centrifuged at 800 × *g* for 10 min to pellet the charcoal; aliquots (600 μL) were taken and the radioactive counting was determined. The IC_{50} of each compound was calculated according to the plots of concentration versus percentage of binding.

2.5. In vivo experiments

*2.5.1. Determination of the effect of **2**–**4** on the number of corpus lutea present in the ovary and on the endometrial thickness with a secretory activity from treated mice*

The animals were kept in a room with controlled temperature (22 °C) and light–dark periods of 12 h. Food and water were provided *ad libitum*.

In order to know the number of corpus lutea present in the ovary we used random cycling mature females CD1 Mice (20–25 g) which were synchronized for estrus with 2 μg of luteinizing hormone-releasing hormone (Sigma) (in phosphate-buffered saline containing 0.1% of bovine serum albumin) administered subcutaneously per mouse at 9:00 h and again at 16:00 h [8]. Animals were allowed to rest for 8 days before the administration of the test compounds; then they were grouped, with four mice per treatment. In the morning of the 9th day following luteinizing hormone-releasing hormone treatment, the mice of three different groups, were injected with compounds **2**–**4** with RBAs of 100% (0.22 mg/kg in 100 μL of sesame oil) once daily subcutaneously for four consecutive days. One vehicle-treated group was maintained as a control. The animals were sacrificed in the morning following the last treatment. The ovaries and the uteri of mice of each group were removed and prepared for histological examination. Tissues were fixed with buffered formaldehyde (10%) [18], processed with conventional histological techniques and included in Paraplast (Oxford Labware, St. Louis, MO 63103, USA). The ovary from each group of mice was cut sagittally, whereas the uteri were cut transversally; 5 μm serial sections of the included tissues were obtained. These sections were stained using hematoxylin–eosin [19] and analyzed under a clear field light microscope (Axioskope II, Carl Zeiss) and image analyzer (Axiovision 4.5, Carl Zeiss). The corpus lutea present in the ovary of each animal/group were counted. The results were analyzed using one-way analysis of variance and Dunnett's Method to compare means, with JMP IN 5.1 software. Micrographs of the specimen were taken with an AxioCamMRc5 (Carl Zeiss).

3. Results

*3.1. Synthesis of steroidal derivatives **2**–**4***

Compounds **5**–**11** were prepared from the commercially available 16-dehydropregnenolone acetate **5** [10] (Fig. 2). The final products **2**–**4** were obtained by esterification of the free alcohol **11** with the corresponding acid. The NMR, UV, IR and mass spectra of all intermediates and final products confirmed unequivocally the structure of these compounds.

3.2. Relative binding affinities of the homoandrostadiene derivatives to the PR

We evaluated three new steroidal compounds: **2** without fluorine atom in the ester side chain, **3** having a fluorine atom in ortho position in the side chain and **4** with a fluorine atom in meta position in the side chain for the inhibition of [^{3}H] R5020 binding to the progesterone receptor. The "IC_{50} values" for the displacement of [^{3}H] R5020 binding to the progesterone receptor and RBAs are shown in Table 1. Progesterone and R5020 as well as steroids **2**–**4** have similar IC_{50} values; these steroids bind to the progesterone receptor with RBAs of 100%.

On the other hand RU486 also binds to the PR with an IC_{50} value of 1.39 nM and a RBA value of 28.7% (Table 1). This compound has an affinity for the PR as previously had been reported [7].

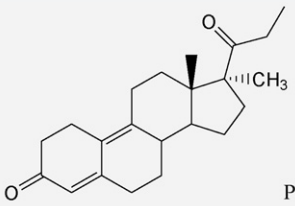
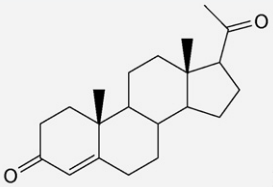
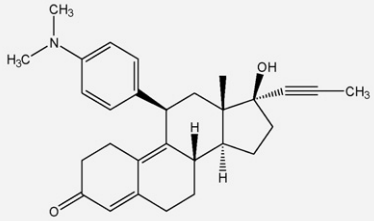
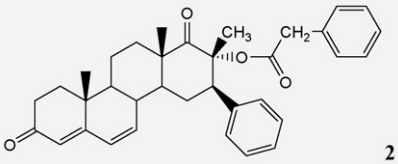
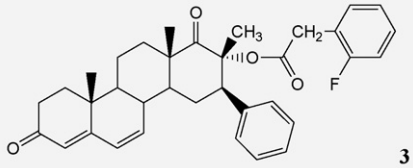
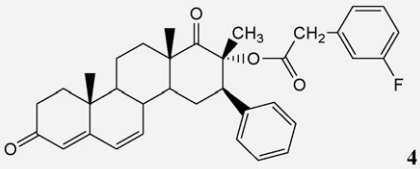
Having demonstrated in this study that the novel steroids bind to the PR, we also evaluated their binding to the AR [12]. It was shown that the tested steroids did not bind to the AR since none of the steroids inhibited the [^{3}H] mibolerone binding to the AR present in the cytosol obtained from castrated rats. Cold (not radioactive) mibolerone inhibited the binding to the AR at concentration of 6 nM.

3.3. In vivo experiments

*3.3.1. Effect of **2**–**4** in the number of corpus lutea in the ovary of mice*

After 13 days following luteinizing hormone-releasing hormone treatment, the mice of the control group showed the presence of 14 ± 4 corpus lutea, whereas the animals treated with steroids **2**–**4** with RBAs of 100%, had 11 ± 7, 12 ± 2, and 10 ± 4 respectively

Table 1
Relative binding affinities (RBAs) of the novel steroidal compounds **2**–**4** to the progesterone receptor.

Structures	IC_{50} (nM)	RBA (%)
 Promegestone (R5020)	0.37 ± 0.065	100
 Progesterone	0.36 ± 0.045	100
 Mifepristone (RU486)	1.39 ± 0.19	28.7
 **2**	0.37 ± 0.011	100
 **3**	0.37 ± 0.089	100
 **4**	0.37 ± 0.036	100

IC_{50} ± standard deviations and RBA values of the novel steroidal compounds to the progesterone receptor obtained from rabbits' uteri, previously treated with estrogens. Each experiment was carried out in two different times by duplicate. The RBA was calculated according to the following equation: RBA = (IC_{50} of [^{3}H] promegestone/IC_{50} of inhibitor) × 100.

(Fig. 3). It is evident from this study that the novel steroids **2**–**4** did not significantly inhibit the ovulation. On the other hand, no degenerative change was observed in the ovary of the mice treated with steroids **2**–**4**.

3.3.2. *Mice uterine transformations*

After 13 days following luteinizing hormone-releasing hormone treatment, the uterus specimens of the mice of the control group, showed an enlargement of the endometrial thickness, which is characteristic of the progestational phase. An intense secretory activity and an increase of the luminal folding of the endometrium were also observed, both indicating a progestational activity. However, the endometrium of the mice treated with steroids **2**–**4** (with RBAs of 100%), showed a reverse progesterone-induced transformation effect, with no enlargement of the endometrial thickness. Furthermore, we also observed a large reduction of the secretory activity and luminal folding in the endometrium of the treated mice, thus indicating that compounds **2**–**4** have antagonistic effect for this tissue. On the other hand, we also observed a reduction of the diameter of the uteri of the treated mice compared to the con-

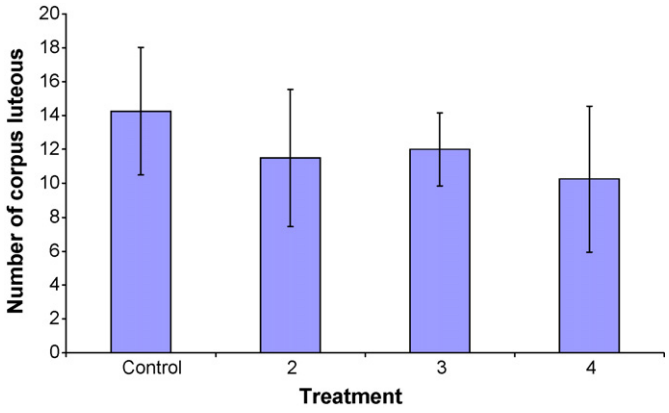


Fig. 3. Number of corpus lutea present in the ovary of mice, after 8 days of treatment with 2 μg of LHRH. The mice were treated daily for 4 days with vehicle (controls) or with the novel steroids. No significant differences were observed between the control and treated groups.

trol group. The pictures of the histological sections are shown in Figs. 4 and 5.

4. Discussion

In this paper, we showed that steroids **2**–**4** bind to the PR present in the cytosol from estrogen-primed rabbits. However, these compounds did not bind to the AR existing in the cytosol of rat prostate.

Progesterone, promegestone as well as steroids **2**–**4** showed similar IC_{50} values and RBAs of 100%. These data indicated that the studied compounds had high affinity for the PR. Steroids **2**–**4** also showed a higher affinity for the PR than RU486.

Since the binding assay showed that the concentration of the unlabeled promegestone necessary for the displacement of 50% of the labeled promegestone (0.4 nM) used for the binding to PR was 0.4 nM, this fact indicates a high reliability for this assay. The competition studies previously reported by Palmer et al. [16] showed an IC_{50} value for the unlabeled progesterone of 4 nM, when they used labeled promegestone in a concentration of 0.4 nM. The difference between Palmer's and our studies is that we could precipitate the PR with ammonium sulfate [8]; this procedure permits the formation of a purified fraction of the PR [17] and better competition analysis.

On the other hand the competition study reported by Chandrasekhar and Amstrong [20] showed a RBA value of Mifepristone (RU486), for the PR, of 187.5%. However, in our method, which uses ammonium sulfate for the precipitation of the PR from primed estrogen rabbit uterus, we found a RBA value for RU486 of 28.7%. This difference in the RBA value could be explained on the ground that Chandrasekhar and Amstrong's method did not use purified fraction of the PR. These authors used labeled promegestone in a concentration of 10 nM; however they needed 180 nM of unlabeled promegestone for the displacement of 50% of the labeled promegestone (10 nM). These data demonstrated that the use ammonium sulfate for the precipitation of PR is an advantage. Gestrinone with a RBA value of 75% [21] is another steroidal antiprogestin, which had

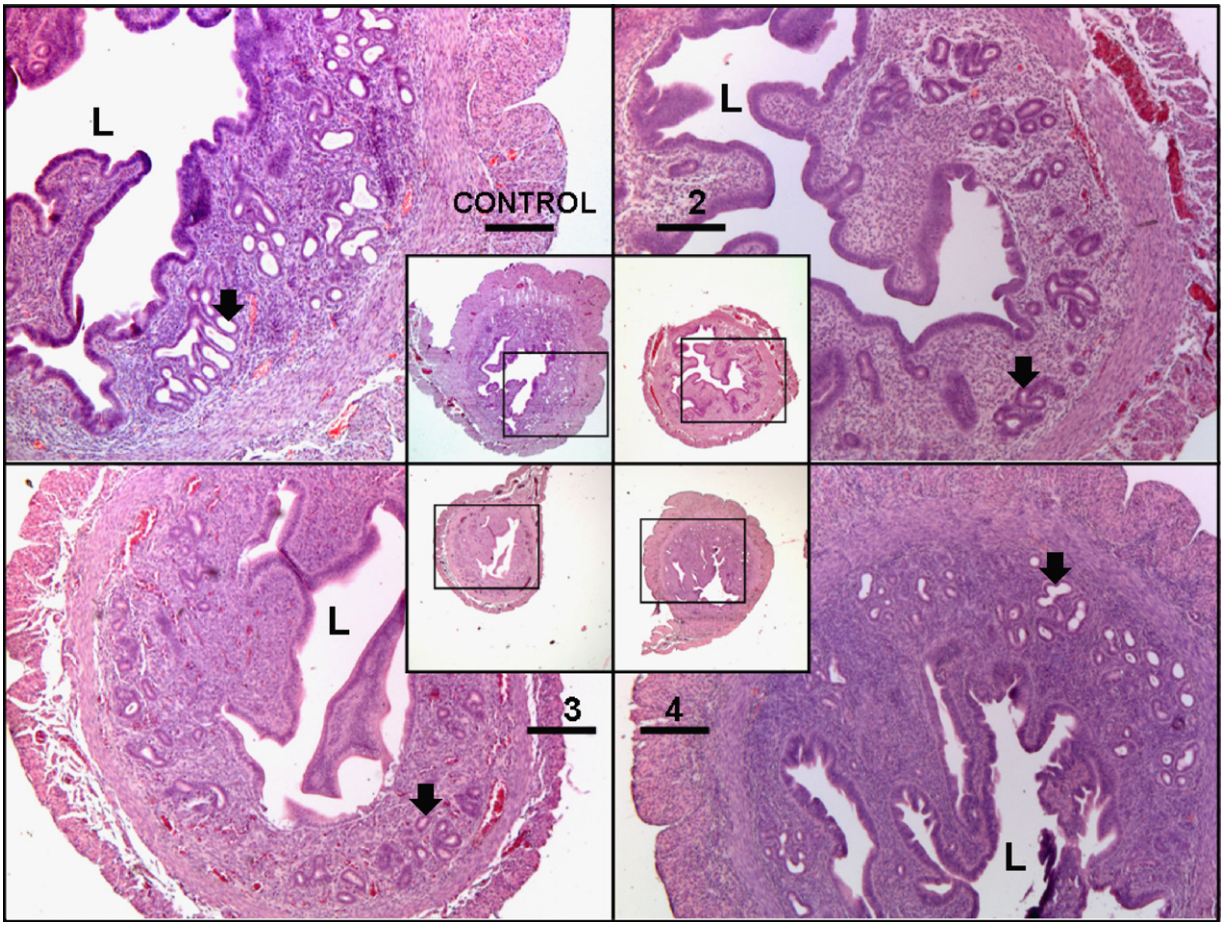


Fig. 4. These photomicrographs show the histology of the mice's uteri. Important changes can be observed. In the center are shown transverse sections of control and experimental groups (**2**–**4**) (H–E, bar 1000 μm). In photomicrographs 2-4 can be observed an evident reduction of the uterus' diameter and lumen (L), when compared to the control group. Framed regions were enlarged to show endometrial glands (arrow). Experimental groups exhibit differences as compare to the control group (H–E, bar 200 μm).

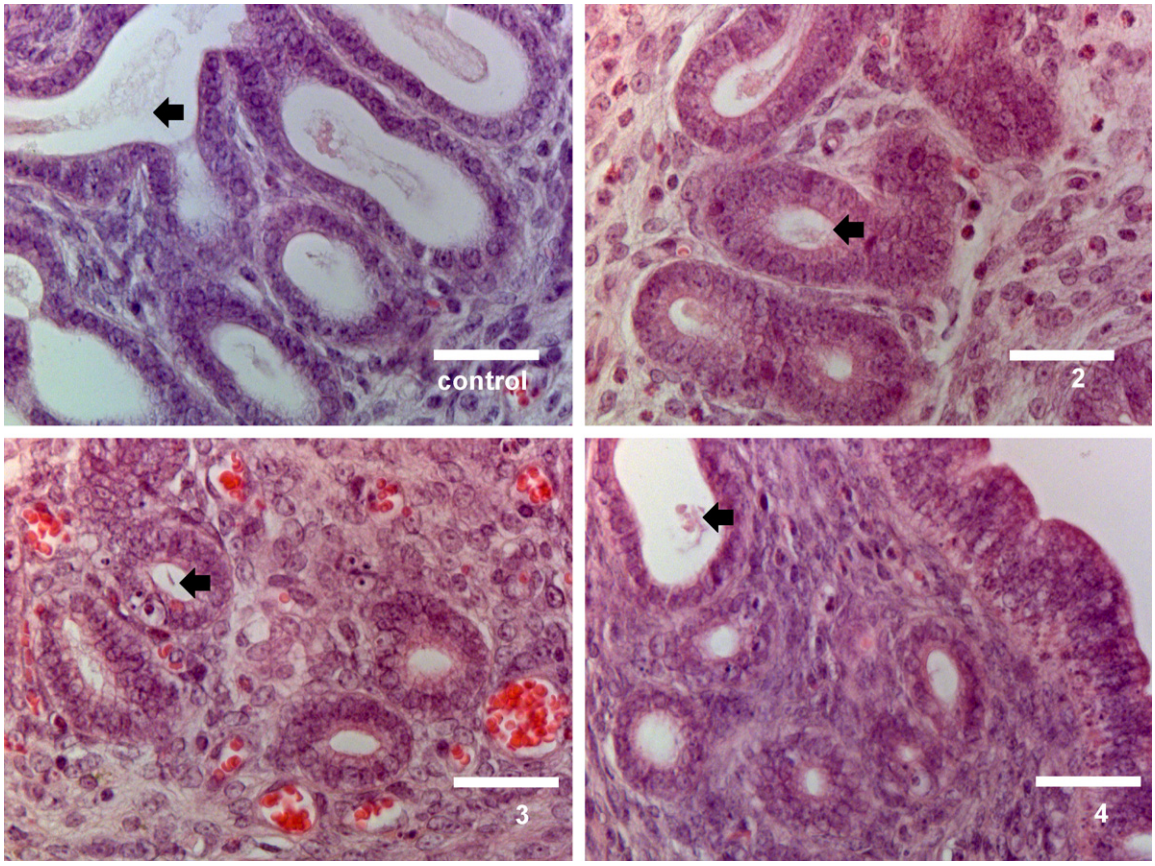


Fig. 5. These photomicrographs show the histology of the mice's uteri. Experimental groups (photomicrographs **2**–**4**) show a reduction in the number and activity of endometrial glands as manifested by the size and dilatation when compared to the control; an abundant secretion can be observed, arrows (H–E bar 40 μm).

been used for the treatment of endometriosis. This steroid demonstrated a weak agonistic activity and a marked pituitary inhibitory activity.

The fact that **2**–**4** did not bind to the AR could be advantageous for its use in the control of fertility (menses-induction and abortion) [7,22], in hormone dependent tumors (e.g. breast cancer) and the control of cell growth and differentiation (endometriosis [5,21]) because they will not produce an androgenic effect.

It has been shown by several authors [7,23–28] that endocrine bioassays using the uterine transformations assay, as parameter for the determination of progestational activity is a reliable method for the *in vivo* evaluation. This assay gives more real information than those carried out *in vitro* and can be used as a definite test. Previously, it had been demonstrated that progesterone antagonists such as mifepristone have reverse progesterone-induced transformation in estrogen-primed rabbit uterus [25] and later this compound was successfully used for the treatment of endometriosis [21,29].

The novel steroids, **2**–**4**, inhibited progesterone-stimulated uterine transformations, in the mice treated with luteinizing hormone-releasing hormone. Therefore, these compounds could be considered as antagonists for the PR present in the mouse uterus. These results indicated also that **2**–**4**, produced a similar effect in the mice uterus as previously reported for mifepristone in the rabbit uterus [24]. On the other hand **2**–**4** failed to inhibit the ovulation. The effect of the antiprogestin mifepristone to inhibit the ovulation has been reported in the past [29].

Compounds **2**–**4** impair luteal phase endometrial development and did not inhibit the ovulation.

The histological analysis of the ovary is a reliable way to determine the ovulation and the existence of a degenerative process in this tissue, produced by the new compounds. This method offers an advantage over the previously reported results [8]. The histological analysis of the ovary in the treated animals indicated that **2**–**4** did not produce any degenerative process in the ovary.

The results from this study indicate very clearly that these steroids showed a high binding affinity as well as high antagonistic activity for the PR present in the estrogen-primed rabbit uterus. In view of the fact that they had high potency *in vivo*, they could probably be used for the treatment of endometriosis without suppressing the ovulation, an important improvement as compared to the presently used compounds.

The overall data obtained from this study indicate that the PR antagonists: **2**–**4** (*D*-homoandrostadienone-derivatives) did not exhibit androgenic effects, since they did not bind to the AR.

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