Selective synthesis of the two main progesterone metabolites, 3α-hydroxy-5α-pregnanolone (allopregnanolone) and 3α-hydroxypregn-4-en-20-one, and an assessment of their effect on proliferation of hormone-dependent human breast cancer cells

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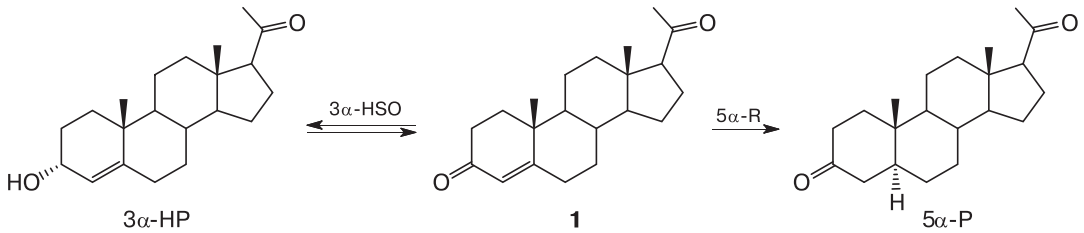
A directed synthesis of two progesterone metabolites, allopregnanolone and 3α-hydroxypregn-4-en-20-one, from Δ^{16}-pregnanolone and progesterone, respectively, was carried out by a reduction of the carbonyl groups in positions 3 and subsequent inversion of the configuration of the resulting alcohols by the Mitsunobu reaction. The selectivity of the reduction of the conjugated carbonyl group in position 3 of progesterone with sodium borohydride in the presence of cerium(III) chloride (Luche reduction) was demonstrated. The effect of the obtained metabolites on the proliferation of breast cancer cells of the MCF-7 and T47D lines under normal and steroid-free conditions was studied. It is shown that the effect of these compounds on the proliferation depends on the presence of additional steroids in the culture medium. Metabolites exerted small cytostatic effects on the growth of the MCF-7 cells under standard conditions, while the transfer of the cells to a steroid-free medium weakened these cytotoxic effects. In the experiments with the T47D line cells, the cell growth was stimulated under both standard and steroid-free conditions. Allopregnanolone and progesterone stimulate the growth to a greater extent under steroid-free conditions than under standard ones.

Key words: progesterone, allopregnanolone, 3α-hydroxypregn-4-en-20-one, steroid metabolites, cytotoxicity, luminal cancer, Luche reduction, MTT assay.

Steroid hormones belong to the main regulators of animal and human vital activity. The natural hormone progesterone (**1**) plays an essential role in the female body, regulating ovulation, preparation of the endometrium to implantation, pregnancy preservation, and many other physiological functions.[1] It is known that progesterone quickly transforms into various metabolites in different body tissues, and these metabolites can have specific physiological effects, thereby affecting the efficiency of the hormone. The main metabolites are 5α- and 5β-derivatives with reduced 4,5-double bonds, 20-dihydro derivatives, 5α(β)-pregnan-3α(β)-ol-20-ones, and their 20-dihydroxy derivatives, 5α(β)-pregnan-3,20-diols.[2,3] In particular, two main metabolites of progesterone in the human mammary gland tissue are 3α-hydroxyprogesterone (3α-HP) and 5α-dihydroprogesterone (5α-P).[4] The process of metabolism of progesterone (**1**) in the human mammary gland tissue under the influence of 3α-hydroxysteroid oxidoreductase (3α-HSO) and 5α-reductase (5α-R), which leads to 3α-HP and 5α-P, is shown[5] in Scheme 1.

Scheme 1



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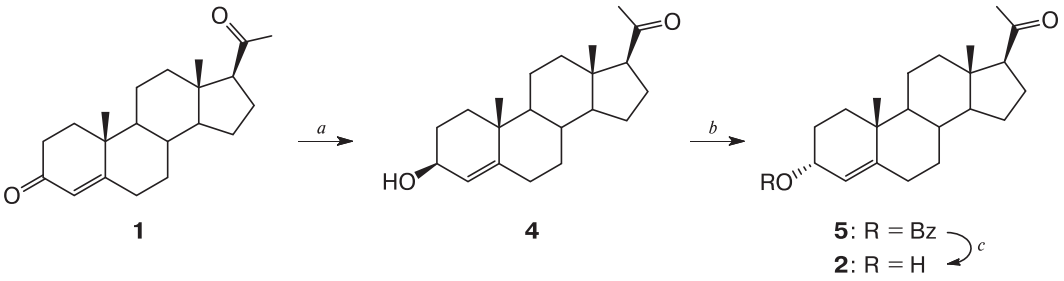
Scheme 2



Reagents and conditions: *a*. $NaBH_4$, $CeCl_3 \cdot 6H_2O$, MeOH, −20 °C. *b*. BzOH, $PyPPh_2$, diisopropyl azodicarboxylate (DIAD), toluene, 80 °C. *c*. KOH—MeOH.

In the early 2000s, it was revealed that in the normal tissue of a human mammary gland, progesterone is metabolized mainly with participation of reversible 20α- and 3α-hydroxysteroid oxidoreductases, resulting in the 4-pregnene derivatives, *viz.*, 20α- and 3α-hydroxyprogesterones, respectively. These metabolites have valuable biological properties, exhibiting antiproliferative effect. The metabolism of progesterone in a tumor significantly differs from that in the normal tissue. In malignant cells, progesterone is predominantly metabolized by 5α-reductase to a 5α-reduced metabolite exhibiting undesirable proliferative activity in a breast tumor.[4,6—8]

Recently we studied biotransformation of pentacyclic progesterone analogs *in vivo* and showed that their metabolic pathways are identical to those of endogenous progesterone.[9] In continuation of our research in the field of the synthesis and investigation of biological effects of progestins and their metabolites, we have carried out a selective synthesis of two progesterone metabolites, *viz.*, 3α-hydroxypregn-4-en-20-one (**2**) and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) (**3**), and studied their effect on proliferation of the T47D and MCF-7 cells of hormone-dependent human breast cancer. Metabolites **2** and **3** were isolated earlier[10] in low yields (15 and 30%) from products of reduction of progesterone and 5α-pregnan-3,20-dione, respectively, with KS-selectride.

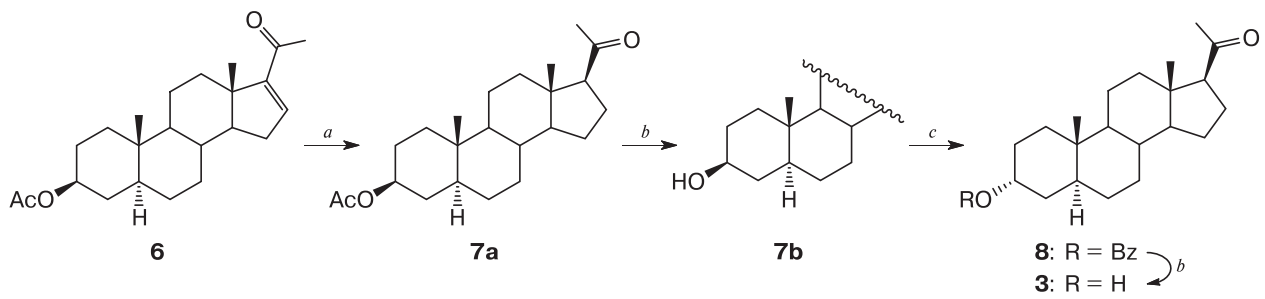
Results and Discussion

We have developed a convenient preparative synthesis of 3α-hydroxypregn-4-en-20-one (**2**) by a selective reduction of the Δ^4-3-keto group of progesterone (**1**) according to the Luche method[11] by the action of $NaBH_4$ in methanol in the presence of $CeCl_3 \cdot 6H_2O$ followed by the inversion of the center in position 3 of the formed steroid **4** by the Mitsunobu reaction. At the stage of preparation of benzoate **5**, diphenyl(2-pyridyl)phosphine[12] was used instead of triphenylphosphine to achieve more efficient removal of unreacted phosphine and its oxide by simple washing with diluted hydrochloric acid. The desired 3α-hydroxypregn-4-en-20-one (**2**) was obtained by alkaline hydrolysis of benzoate **5** with methanol solution of potassium hydroxide (Scheme 2).

3α-Hydroxy-5α-pregnan-20-one (**3**) was obtained from Δ^{16}-pregnanolone acetate (**6**) (Scheme 3). The double bond in the molecule of steroid **6** was quantitatively hydrogenated in the presence of 10% Pd/C, then the 3-acetoxyl group in obtained compound **7a** was hydrolyzed, producing 3β-hydroxysteroid **7b**, which resulted in the formation of 3α-hydroxypregnane **3** in high yield under the conditions of the Mitsunobu reaction (see Scheme 3).

We have studied the influence of the prepared compounds **2** and **3**, as well as progesterone **1** on the growth

Scheme 3



Reagents and conditions: *a*. H_2, Pd/C. *b*. KOH—MeOH. *c*. BzOH, $PyPPh_2$, DIAD, toluene, 80 °C.

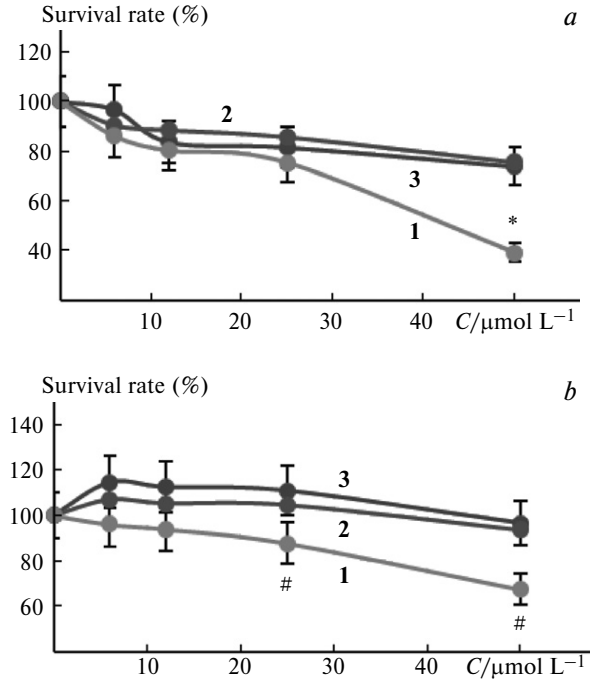


Fig. 1. The effect of progesterone (**1**) and its metabolites **2** and **3** on the survival rate of the MCF-7 breast cancer cells in 72 h of incubation under the standard (*a*) and steroid-free (*b*) conditions (for purification of the serum from steroids see the Experimental). Symbols "*" and "#" correspond to $p < 0.05$ in comparisons of progesterone with compounds **2** and **3**.

of cells of hormone-dependent breast cancer. The experiments were carried out with two lines of luminal breast cancer cells: MCF-7 and T47D. Progesterone **1** and its metabolites **2** and **3** in concentrations up to 25 μmol L^{-1} did not cause significant cytostatic or stimulating effects on the MCF-7 cells (Fig. 1, *a*). At steroid concentrations of 25 μmol L^{-1}, the cell growth inhibition did not exceed 25% compared to the control cells. Upon going to higher concentrations (50 μmol L^{-1}), the cytostatic effect of progesterone became more pronounced than that of its metabolites **2** and **3**. Under these conditions, 3α-hydroxypregn-4-en-20-on (**2**) and allopregnanolone (**3**) inhibited the MCF-7 cell growth by less than 30%, whereas progesterone (**1**) resulted in the 60% proliferation suppression.

Incubation of cells with a studied compound for 72 h in a medium with 7—10% fetal serum is the generally accepted standard for preclinical experiments. These experiments are usually aimed at characterization of antiproliferative potential of newly developed drugs or assessment of the capability of physiological metabolites to promote proliferation of malignant cells or normal epithelium. Universal protocols of MTT assay and other analogous procedures make it possible to compare data obtained in different laboratories. For a more detailed characterization of steroids, serum purified from steroids (steroid-free conditions) is additionally used.[13] Under the steroid-free conditions, it is possible to model direct competition between two certain steroids or to investigate the effect of particular steroid on a hormone receptor without the background created by endogenous serum steroids. Moreover, the steroid-free conditions can be considered as an adequate model for studying tumors in menopausal women (when the steroid hormone level is decreased). The steroid-free conditions make it possible to estimate the ability of non-steroid molecules to act as potential agonists of hormone receptors. For instance, it was demonstrated that clodronic acid, which is a clinically approved bone resorption inhibitor, exhibits estrogenic effects.[13] Thus, the use of steroid-free conditions expand the possibilities of *in vitro* studies of hormone metabolites and compounds with hormone-like properties.

The MCF-7 cells were transferred to steroid-free conditions (see the Experimental). It was shown the steroid-free conditions weaken the cytostatic effect of progesterone (**1**) and completely neutralize the effects of metabolites **2** and **3** (see Fig. 1, *b*). Moreover, weak stimulating effect on proliferation of MCF-7 cells was revealed under the steroid-free conditions for compound **3** in low concentrations (15% in comparison with the control cells).

The T47D cells, like the MCF-7 cells, belong to luminal cancer cells. This type of mammary gland tumors is characterized by expression of estrogen and progesterone receptors and a relatively good clinical prognosis. One of differences between these lines consists in higher expression of progesterone receptors in the T47D cells compared to the MCF-7 cells. We revealed a minor stimulating effect of allopregnanolone (**3**) in a concentration of 12 μmol L^{-1} on growth of the T47D cells under standard conditions of culturing (Fig. 2, *a*). Like in the experiments with the MCF-7 cells, progesterone (**1**) in high concentrations caused cytostatic influence on the T47D cells. When the steroid-free conditions were used, it was found that this compound stimulates the T47D cell growth (see Fig. 2, *b*). Low (up to 25 μmol L^{-1}) concentrations of allopregnanolone (**3**) and progesterone (**1**) caused substantial stimulation of cell growth. The effect of metabolite **2** on the cell growth stimulation was less pronounced. In a concentration of 50 μmol L^{-1}, the stimulating effect of compound **3** decreased, whereas progesterone in the same concentration inhibited the cell growth by 44%.

It was shown earlier[5] that in mammary gland tissues, progesterone practically completely transforms into metabolites, the major of which are 5α-P and 3α-HP (see Scheme 1). For both cell lines, metabolite 5α-P significantly prevails over other progesterone metabolites.[14,15] However, in the MCF-10A cells of normal epithelium of mammary gland, progesterone is metabolized to a lesser extent than in tumor cells, and the 5α-P : 3α-HP ratio is smaller by 10—30 times than in the latter.[14,16]

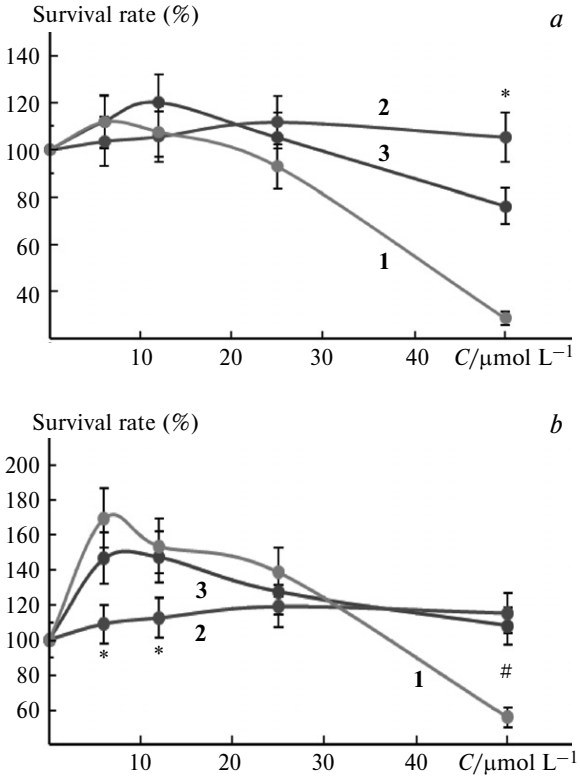


Fig. 2. The effect of progesterone (**1**) and its metabolites **2** and **3** on the survival rate of the T47D breast cancer cells in 72 h of incubation under the standard (*a*) (symbol "*" corresponds to $p < 0.05$ in pairwise comparison of the compounds) and steroid-free (*b*) conditions (symbol "*" corresponds to $p < 0.05$ in comparisons of progesterone with compounds **3** and **1**, symbol "#" corresponds to $p < 0.05$ in comparisons of compound **1** with compounds **2** and **3**).

At the same time it was noted that metabolites 5α-P and 3α-HP themselves have the opposite effect on proliferation of cells of both tumor and normal epithelium of mammary gland: 5α-P considerably stimulates proliferation, while 3α-HP inhibits it.[4,6] Recently[17] it was suggested that the effects of metabolites 5α-P and 3α-HP are mediated by corresponding membrane-associated receptors, however the mechanism of the opposite action of these metabolites at the cellular level has not yet been established. Our experimental data partly support the effects described in the mentioned studies. Steroids **2** and **3** had small cytostatic effects on the growth of the MCF-7 cells of breast cancer under the standard conditions, and no significant difference between the actions of these compounds was found. Progesterone in a concentration of 50 μmol L^{-1} was more active than the synthesized metabolites. The use of steroid-free conditions weakened the cytostatic effects of the compounds, and a tendency to stimulation of growth of the MCF-7 cells was noted for allopregnanolone.

In the experiments with the luminal breast cancer cells of another line (T47D), more pronounced stimulation of their growth was found. Almost no cytostatic effect was observed under the standard conditions for the compounds under study. Under the steroid-free conditions of culturing, progesterone (**1**) and allopregnanolone (**3**) exhibited marked stimulating influence on the cell growth, while the stimulating effect of compound **2** was not exceeded 20%. Unfortunately, the 5α-P and 3α-HP concentration dependences shown in an earlier study[7] correspond to values not exceeding 1 μmol L^{-1}, which limits the possibility of a direct comparison of those data with ours. The authors studied the effect of 5α-P and 3α-HP on incorporation of tritium-labeled thymidine in DNA. This method is not a direct analog of the MTT assay, which provides an additional explanation of partial discrepancy between the data obtained for 3α-HP.

Thus, even inside the cells of breast cancer of luminal type, the action of progesterone metabolites can vary. In particular, their influence on proliferation depends on the presence of additional steroids in the medium for culturing. The transition to steroid-free conditions in the case of MCF-7 decreases the cytostatic effect of metabolites **2** and **3**, whereas in the case of the T47D cells, it enhances the capability of allopregnanolone (**3**) and progesterone (**1**) to stimulate the cancer growth.

On the whole, metabolism of progesterone (and synthetic progestins as anticancer drugs) is a highly important factor of progression of breast tumor. Further more detailed studies of the status of progesterone receptors and the activity of progestin metabolism enzymes are needed to correct the hormonal treatment of patients with breast cancer. Patients with breast cancer of luminal type with high level of progesterone receptors or/and progestin metabolism enzymes can be singled out into a separate group for the creation of personalized treatment protocols.

Experimental

Melting points were obtained using a Boetius melting-point apparatus. Specific optical rotation was measured using a JASCO P-2000 digital polarimeter in $CHCl_3$ at 22 °C. ^1H NMR spectra were recorded in $CDCl_3$ at 30 °C with the use of a Bruker AM-300 spectrometer (300.13 MHz). The signal of residual $CHCl_3$ (δ_H 7.27) in $CDCl_3$ was used as a standard. High resolution mass spectra were obtained with a Bruker micrOTOF II mass spectrometer using electrospray ionization (ESI). Analytical TLC was carried out on Silica gel 60 F_{254} plates (Merck). Compounds were revealed by the treatment of the plates with 1% solution of $Ce(SO_4)_2$ in 10% aqueous H_2SO_4 and subsequent warming up. The preparative separation was carried out by column chromatography using a column with Kieselgel 60 (0.063—0.200 μm) silica gel (Merck) at the compound : sorbent ratio = 1 : 40. Solvents were purified according to standard procedures. Yields of products are given for recrystallized samples. Starting 3β-acetoxy-5α-pregn-16-en-20-one was kindly provided by G. S. Grinenko (All-Union Scientific Research Chemical-Pharmaceutical Institute), progesterone was obtained from Fisher, other reagents were obtained from Sigma.

3β-Hydroxypregn-4-en-20-one (4). A solution of progesterone (**1**) (314 mg, 1 mmol) and $CeCl_3 \cdot 6H_2O$ (374 mg, 1 mmol) in methanol (10 mL) was cooled to −20 °C, then $NaBH_4$ (19 mg, 0.5 mmol) was added under stirring. In 10 min, acetone (0.37 mL) was added to the mixture, and cooling was ceased. The reaction mixture was poured into cold water (30 mL) and extracted with ethyl acetate (3×10 mL). The organic layer was washed with a saturated NaCl solution (15 mL) and dried over anhydrous Na_2SO_4. The obtained product was chromatographed on a column. By elution with CH_2Cl_2—petroleum ether mixtures (2 : 1) with additions of 0.2→1% acetone, 3β-hydroxypregn-4-en-20-one **4** was obtained (210 mg, 67%), m.p. 153—155 °C (see Ref. 18: m.p. 159 °C). ^{1}H NMR, δ: 0.66 (s, 3 H, C(18)H_3); 1.07 (s, 3 H, C(19)H_3); 2.13 (s, 3 H, C(21)H_3); 2.50—2.55 (m, 1 H, H(17)); 4.17 (br.s, 1 H, H(3)); 5.31 (s, 1 H, H(4)) (see Ref. 10).

3α-Benzoyloxypregn-4-en-20-one (5). A solution of DIAD (0.36 mL, 1.7 mmol) in dry toluene (1.8 mL) was added dropwise to a cooled (ice bath) mixture of alcohol **4** (350 mg, 1.1 mmol), benzoic acid (212 mg, 1.7 mmol), and diphenyl(2-pyridyl)phosphine (457 mg, 1.7 mmol) in toluene (8 mL). The reaction mixture was heated at 80 °C for 16 h, then cooled to room temperature and washed with an 1 *N* solution of HCl (4×10 mL), a saturated $NaHCO_3$ solution (3×10 mL), and a saturated NaCl solution (10 мл). The organic fraction was separated, dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The rest was chromatographed on a column with silica gel. 3α-Benzoyloxypregn-4-en-20-one (**5**) was obtained in the form of dense oil (330 mg, 70%) by elution with CH_2Cl_2—petroleum ether mixtures (2 : 1) with additions of 0.2→1% acetone. The product was further used without additional purification. ^{1}H NMR, δ: 0.68 (s, 3 H, C(18)H_3); 1.12 (s, 3 H, C(19)H_3); 2.13 (s, 3 H, C(21)H_3); 2.55—2.59 (m, 1 H, H(17)); 5.39 (br.s, 1 H, H(3)); 5.59 (s, 1 H, H(4)); 7.49 (t, 2 H, Ph, J = 7.7 Hz); 7.59 (t, 1 H, Ph, J = 7.4 Hz); 8.09 (d, 2 H, Ph, J = 7.2 Hz).

3α-Hydroxypregn-4-en-20-one (2). A suspension of benzoate **5** (330 mg, 0.78 mmol) and a 3 *N* KOH solution (1.3 mL) in MeOH (6 mL) was heated at 60 °C for 2.5 h. The reaction mixture was poured out into cold water (23 mL) acidified with concentrated HCl to pH 1—2 and extracted with ethyl acetate (3×10 mL). The organic layer was washed with a saturated $NaHCO_3$ solution (15 mL) and dried over anhydrous Na_2SO_4, the solvent was then removed in vacuo. The rest was chromatographed on a silica gel column. After elution with CH_2Cl_2—petroleum ether mixtures (2 : 1) containing 0.2→1% acetone and recrystallization, 3α-hydroxypregn-4-en-20-one (**2**) was obtained (150 mg, 64%), m.p. 127—129 °C (hexane—acetone) (see Ref. 10: m.p. 127—129 °C), $[\alpha]_D^{22}$ +229 (*c* 1.00) (see Ref. 10: $[\alpha]_D^{26}$ +231 (*c* 1.80, $CHCl_3$)). ^{1}H NMR, δ: 0.65 (s, 3 H, C(18)H_3); 0.99 (s, 3 H, C(19)H_3); 2.12 (s, 3 H, C(21)H_3); 2.51—2.55 (m, 1 H, H(17)); 4.08 (br.s, 1 H, H(3)); 5.48 (d, 1 H, H(4), J = 4.5 Hz) (see Ref. 10). High resolution mass spectrum, found: *m/z* 339.2293 $[M + Na]^+$. $C_{21}H_{32}NaO_2$. Calculated: 339.2295.

3β-Acetoxy-5α-pregnan-20-one (7a). A solution of 3β-acetoxy-5α-pregn-16-en-20-one (**6**) (1.52 g, 4.2 mmol) in dioxane (25 mL) was hydrogenated in the presence of Pd/C (0.07 g, 10%) until complete conversion of the starting compound according to TLC (development with an aqueous solution of $KMnO_4$). The catalyst was filtered, the solvent was evaporated in vacuo, the rest was recrystallized from a hexane—acetone mixture to give 3β-acetoxy-5α-pregnan-20-one (**7a**) (1.5 g, 98%), m.p. 137—142 °C (see Ref. 19: m.p. 146—147 °C). ^{1}H NMR, δ: 0.67 (s, 3 H, C(18)H_3); 0.82 (s, 3 H, C(19)H_3); 1.12—1.17 (m, 1 H, H(5)); 2.10, 2.15 (both s, 2×3 H, 3-OAc, C(21)H_3); 2.50—2.55 (m, 1 H, H(17)); 4.55—4.59 (m, 1 H, H(3)) (see Ref. 19).

3β-Hydroxy-5α-pregnan-20-one (7b). A suspension of acetate **7a** (790 mg, 2.2 mmol) and an 1.8 *N* KOH solution (5.6 mL) in MeOH (22 mL) was heated at 60 °C for 1 h. The reaction mixture was poured out into cold water (40 mL) acidified with concentrated HCl to pH 1—2 and extracted with ethyl acetate (3×10 mL). The organic layer was washed with water (20 mL), dried over anhydrous Na_2SO_4, the solvent was removed in vacuo to give 3β-hydroxysteroid **7b** (670 mg, 96%), m.p. 194—195 °C (ethanol) (see Ref. 20: m.p. 194—195 °C). ^{1}H NMR, δ: 0.68 (s, 3 H, C(18)H_3); 0.82 (s, 3 H, C(19)H_3); 0.99—1.30 (m, 1 H, H(5)); 2.13 (s, 3 H, C(21)H_3); 2.50—2.55 (m, 1 H, H(17)); 3.57—3.63 (m, 1 H, H(3)) (see Ref. 21).

3α-Benzoyloxy-5α-pregnan-20-one (8). A solution of DIAD (0.7 mL, 3.31 mmol) in dry toluene (3.5 mL) was added dropwise to a cooled (ice bath) mixture of alcohol **7b** (670 mg, 2.10 mmol), benzoic acid (404 mg, 3.31 mmol), and diphenyl(2-pyridyl) phosphine (876 mg, 3.33 mmol) in toluene (17 mL). The reaction mixture was heated at 80 °C for 16 h, cooled to room temperature, and washed with an 1 *N* HCl solution (4×10 mL), a saturated $NaHCO_3$ solution (3×10 mL), and a saturated NaCl solution (10 mL). The organic fraction was separated and dried over anhydrous Na_2SO_4, the solvent was removed in vacuo. The rest was chromatographed on a silica gel column. 3α-Benzoyloxy-5α-pregnan-20-one (**8**) was obtained in the form of dense oil (750 mg, 84%) by elution with CH_2Cl_2—petroleum ether mixtures (1 : 1) containing 0.2→1% acetone and was used further without additional purification. ^{1}H NMR, δ: 0.64 (s, 3 H, C(18)H_3); 0.87 (s, 3 H, C(19)H_3); 2.13 (s, 3 H, C(21)H_3); 2.55—2.59 (m, 1 H, H(17)); 5.32 (br.s, 1 H, H(3)); 7.48 (t, 2 H, Ph, J = 7.7 Hz); 7.58 (t, 1 H, Ph, J = 7.4 Hz); 8.09 (d, 2 H, Ph, J = 7.2 Hz).

Hydroxy-5α-pregnan-20-one (allopregnanolone) (3). A suspension of benzoate **8** (750 mg, 1.77 mmol) and a 7 *N* KOH solution (2.3 mL) in MeOH (45 mL) was heated at 60 °C for 2.5 h. The reaction mixture was poured into cold water (37 mL) acidified with concentrated HCl to pH 1—2 and extracted with ethyl acetate (3×10 mL). The organic layer was washed with a saturated $NaHCO_3$ solution (15 mL), water (15 mL), and dried over anhydrous Na_2SO_4, the solvent was removed in vacuo. The rest was chromatographed on a column with silica gel. Allopregnanolone (**3**) was obtained (320 mg, 57%) by elution with CH_2Cl_2—petroleum ether mixtures (1 : 1)) containing 0.2→1% acetone, m.p. 177 °C (ethanol) (see Ref. 22: m.p. 174—176 °C), $[\alpha]_D^{22}$ +103.9 (*c* 1.00) (see Ref. 23: $[\alpha]_D^{16}$ +96 (*c* 0.5, $CHCl_3$)). ^{1}H NMR, δ: 0.62 (s, 3 H, C(18)H_3); 0.80 (s, 3 H, C(19)H_3); 2.13 (s, 3 H, C(21)H_3); 2.52—2.61 (m, 1 H, H(17)); 4.07 (br.s, 1 H, H(3)) (see Ref. 10).

Biological studies. For biological testing, the compounds were dissolved in DMSO to a concentration of 5 mmol L^{-1} and kept at −20 °C until the use. Human breast cancer cells MCF-7 and T47D (ER- and PR-positive) were obtained from the ATCC collection. The MCF-7 and T47D cells were cultured *in vitro* in the standard DMEM medium (Gibco) with additions of sodium pyruvate (0.1 mg mL^{-1}, ChemCruz). The medium contained a 10% fetal calf serum (HyClone), penicillin (50 IU mL^{-1}), and streptomycin (50 μg mL^{-1}) (PanEco). Incubation was carried out in a 5% CO_2 atmosphere at 37 °C and relative humidity of 80—90% (a NU-5840E CO_2 incubator, NuAir). The effects of steroids which are present in the fetal serum on the activities of

the compounds under study were assessed under steroid-free conditions. In this case, DMEM without addition of phenol red (Gibco) and fetal serum purified with charcoal with dextran were used. The MTT assay was employed to determine the number of surviving cells. It consisted in the transformation of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into crystalline formazan. Cells were seeded in 24-well plates (Corning) by 40000 MCF-7 cells or 60000 T47D cells per one well under the standard conditions and by 50000 MCF-7 cells or 70000 T47D cells per one well under the steroid-free conditions. The use of different seeding densities made it possible to compensate for the difference in their proliferation rates and create the conditions required for comparing the activities of the compounds. The compounds under analysis were added to cells in 12 h. Just before the experiment, the DMSO solutions of the compounds were mixed with the culture medium in separate tubes. Then, the working solutions were transferred into wells with cells. The corresponding amount of the solvent (DMSO) mixed with the culture medium was added to control cells. The volume of the added solvent was equal to 1% of the total volume of the medium for incubation. The concentration of the compounds in the culture medium was varied from 6 to 50 μmol L^{-1}, the content of the organic solvent did not exceed 1%. In 72 h of incubation with an added compound, the medium was separated, and the MTT reagent (AppliChem) was added to cells, and they were incubated for 2 h. Then the cells were lysed in DMSO, and formazan accumulated by them was dissolved by light shaking of the plate. Optical density of the obtained solutions was measured with a MultiScan FC spectrophotometer (ThermoFisher) at 571 nm. The survival rate of the cells treated with the solvent was taken as 100%. Comparison of the compound activities was carried out using the ANOVA statistics realized in the GraphPad program.

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