Synthesis, structutre and biological activity of substituted [16α,17α]cyclopropapregn-4-ene-3,20-diones

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Aryldiazomethanes generated by vacuum thermolysis of arenecarbaldehyde tosylhydrazone sodium salts react stereospecifically with 16-dehydropregnenolone acetate. Subsequent decomposition of the pyrazoline adducts yielded hitherto unknown substituted [16α , 17α]-cyclopropaprogesterones bearing the aryl substituents at the position 3'. Interaction of such cyclopropaprogesterones with progesterone receptor from rat uterine cytosol was studied.

Key words: synthesis, steroids, progesterone, pentaranes, receptor, cyclopropanes, 1,3-dipolar cycloaddition, X-ray diffraction.

Progestins (progesterone analogs) are the synthetic hormones obtained by chemical transformations of the natural steroid skeleton.¹ This approach served for the synthesis of a large variety of highly active progestins widely used in medicine to treat different gynecological diseases, for hormonal contraception, and in hormone replacement therapy. The effective use of progestins is mainly associated with their ability to bind with the progesterone receptors (PR). Selective progesterone receptor ligands are also used in the treatment of hormone-dependent cancer.¹

Among synthetic progestins we synthesized, a series of biologically active progesterone analogs bearing the D carbocycle fused with the D ring, namely, $[16\alpha, 17\alpha]$ cycloalkaprogesterons (pregna- D'_{3-6} -pentaranes) are of special interest.^{2,3} Compared to natural hormone, these compounds show the minimal geometric distortion of the steroid skeleton and have more hydrophobic molecular surface allowing interaction with PR of experimental animals and humans with an affinity comparable with progesterone affinity as well as a symbasis between binding of some of these steroid-PR complexes (formed by hydrogen bonding and hydrophobic interactions) with hormone response elements in DNA and progestational effect *in vivo*.^{2,3}. The study of the relative binding affinity of the steroid-receptor complexes of D'6- and D'3-pentaranes for hormone response elements in DNA revealed (1) complete symbasis of these values for progesterone, D'₆-pentarane and its close homologs and (2) the absence of this symbasis for D'₃-pentarane.⁴ This fact can be explained suggesting the different tertiary conformations of the steroid-PR complexes of D'_{6} - and D'_{3} -pentaranes with the

same receptor. To confirm this assumption, we synthesized a series of substituted $[16\alpha, 17\alpha]$ cyclopropaprogesterones⁵ and studied they binding with PR. The only known pentaranes bearing an additional three-membered cycle D' are $[16\alpha, 17\alpha]$ cyclopropaprogesterone exhibiting high progestational activity^{2–4} and two its spiro-analogs,^{5,6} which biological properties were not studied.

In the framework of our research on the pentarane structure—biological activity relationship, we report herein the synthesis and structural study of novel substituted

[16α , 17α]cyclopropaprogesterones, 3'-phenyl-[16α , 17α]cyclopropapregn-4-ene-3, 20-dione (**1a**) and 3'-(4-fluorophenyl)[16α , 17α]cyclopropapregn-4-ene-3, 20-



dione (1b). The binding of these steroids and specially prepared compounds 4-6 (Table 1) with the uterine cytosol progesterone receptors were also studied.

Compounds **1a** and **1b** were synthesized in four steps from 16-dehydropregnenolone acetate (DPA) (Scheme 1). 1,3-Dipolar cycloaddition of DPA to phenyl- and 4-fluorophenyldiazomethanes generated by vacuum thermolysis of sodium salts of benzaldehyde tosylhydrazone and 4-fluorobenzaldehyde tosylhydrazone⁷ proceeds regio- and stereospecifically to give the corresponding 3'-aryl-substituted [17α , 16α -*c*]pyrazolines **2a** and **2b**. Pyrolysis of compounds **2a**,**b** was carried out in a melt at 180 °C and a vacuum of 20 Torr. Under these conditions, decomposition of pyrazolines occurs with high conversion (>95%) and is accompanied by partial resinification of the reaction mixture. Phenyl-substituted cyclopropasteroids **3a** and

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Entry	Compound	D´(16α,17α)	RBA (%)
1	Progesterone	_	100
2	$3'$ -Phenyl[16 α ,17 α]cyclopropapregn-4-ene-3,20-dione (1a)	CH-Ph	1.8
3	$3' - (4 - Fluorophenyl)[16\alpha, 17\alpha]$ cyclopropapregn-4-ene-3,20-dione (1b)	CH-C ₆ H ₄ -F-4	2.2
4	16α , 17α -Cyclopropaprogesterone (4)	CH ₂	936
5	$[16\alpha, 17\alpha]$ (Spiro[2.2]penta)pregn-4-ene-3,20-dione (5)	>c⊲ _{Me}	122
6	4',4'-Dimethyl[16 α ,17 α](spiro[2.2]penta)pregn-4-ene-3,20-dione ⁴ (6)	>c	4.3

Table 1. Relative binding affinity (RBA) values of studied compounds for progesterone receptor (PR) from rat uterus

3b (see Scheme 1) were isolated by silica gel column chromatography following crystallization from hexane—diethyl ether (isolated yields of 90—95%). Removal of the 3β -acetate group of compounds **3a** and **3b** and subsequent Oppenauer oxidation of the resulted 3β -hydroxy derivatives leads to target compounds **1a** and **1b** in ~80% yields. The structures of compounds synthesized are confirmed by microanalysis data, mass spectroscopy, and ¹H NMR spectroscopy. The X-ray analysis reveals the *cis* orientation of the phenyl group of the fused cyclopropane moiety and 17β -acetyl group of the steroid skeleton (Fig. 1).

 $[16\alpha, 17\alpha]$ (Spiro[2.2])penta)pregn-4-ene-3,20-dione **5** was synthesized in four steps analogously to 4',4'-dimethyl[16\alpha, 17\alpha] (spiro[2.2]penta)pregn-4ene-3,20-dione (**6**)⁵ by 1,3-cycloaddition of DPA to diazacyclopropane generated by thermolysis of *N*-cyclopropyl-*N*-nitrosourea.

To estimate the effect of the substituents at the position 16α , 17α of the progesterone skeleton on binding with PR, the competition experiments were performed (displacement of [³H]progesterone from the protein complexes).⁸ The relative binding affinity (RBA) values of the synthesized compounds relative to binding affinity of natural progesterone (RBA = 100%) are given in Table 1. These

data indicate that the introduction of the cyclopropane fragment into the progesterone 16α , 17α position (compound 4) does not noticeably affect the affinity for PR. Besides, the affinity of spiropentaprogesterone 5 for PR slightly exceeds the affinity of $[16\alpha, 17\alpha]$ cyclopropaprogesterone 4 due apparently to additional hydrophobic binding with the corresponding hormone-binding pocket of PR, which implies an extra space for this substituent. However, the further increase in the volume of the molecule by introduction of two methyl groups (compound 6) dramatically decreases the affinity. In the case of compounds 1a and **1b**, the equatorial 3'-aryl substituent of the cyclopropane ring (roughly in the plane of the steroid skeleton) almost prevents binding of the steroid with PR. It can be assumed that in the case of compounds 1a and 1b, the size of the aryl substituent at the D' ring exceeds the size of the cavity of the ligand-binding site of PR thereby sterically hindering ligand-receptor binding.

In summary, we like to note that, 16α , 17α -substituted cyclopropaprogesterones **1a**,**b**—**6** exhibit significant cytotoxic effect against HeLa cell line (human cervical cancer). The results of this study and a suggested mechanism of growth inhibition of cancer cell are going to be published elsewhere.

Scheme 1



R = H (a), F (b)

Reagents and conditions: *i*. ArCH=NN(Na)Ts, 175 °C, 0.05 Torr, MeOH–CH₂Cl₂; *ii*. 180–200 °C, 20 Torr; *iii*. KOH–MeOH; *iv*. Al(OPrⁱ)₃, cyclohexanone, PhMe, reflux, 2.5 h.



Fig. 1. Crystal structure of $3^{\prime}\beta$ -phenyl[16α , 17α]cyclopropapregn-4-ene-3,20-dione (1a).

Experimental

Melting points were determined on a Boetius apparatus. ¹H NMR spectra were run on a Bruker AM-300 spectrometer (Germany) on working frequency of 300.13 MHz in CDCl₃ at 30 °C. The chemical shifts are given in the δ scale relative to the residual solvent signal (δ 7.27). Mass spectra (EI, 70 eV, direct inlet, 100 °C) were recorded on a Finnigan MAT INCOS 50 instrument. Elemental analyses were performed on a Perkin—Elmer 2400 Series II C,H,N elemental analyzer. TLC was performed on Silica gel 60 F₂₅₄ pre-coated plates (Merck), elution with hexane—acetone or hexane—diethyl ether. The spots of the compounds were visualized by spraying with 1% Ce(SO₄)₂ in 10% aqueous H₂SO₄ followed by heating. The specific rotations were measured on a PU 07 polarimeter (Russia) in CH₂Cl₂ at 27 °C. Column chromatography (Kieselgel 60, 0.063—0.100 µm (Merck), a compound—sorbent ratio of 1 : 40) was used for product isolation.

Commercial tosylhydrazide, benzaldehyde, and 4-fluorobenzaldehyde (Acros) were used as purchased. 16-Dehydropregnenolone acetate (DPA) is available from Sigma. The solvents were purified prior to use according to the standard procedures.

The organic extracts were washed to neutral, dried with $MgSO_4$, and concentrated *in vacuo*.

Single-crystal X-ray diffraction of compound **1a** was performed on a Bruker 1K SMART CCD automated diffractometer (Mo-K α radiation). Crystals are monoclinic, at 120 K a = 9.1768(13) Å, b = 6.6126 (9) Å, c = 17.943(3) Å, $\beta = 96.500(3)^{\circ}$, space group *P*21. Crystal structure was calculated using SHELXTL PLUS program package.⁹ The experimental details are given in Table 2. The atomic coordinates and complete crystallographic data were deposited with the Cambridge Structural Database (CCDC 930437).

1,3-Dipolar cycloaddition of aryldiazomethanes to 16-dehydropregnenolone acetate (general procedure). The 30 mL oneneck flask charged with sodium salt of the corresponding benzaldehyde tosylhydrazone was connected to a cold trap containing a preliminary prepared solution of DPA in methanol dichloromethane (1 : 1, 6 mL). The trap was cooled with liquid nitrogen and the system was carefully evacuated $(5 \cdot 10^{-2} \text{ mbar})$, then the thermolysis was started by slow rising the bath temperature to 175 °C. After completion of pyrolysis (1–1.5 h) and thawing, the content of the trap was stirred, and the obtained red solution was kept in a freezer at -18 °C for 12–15 h. After decolorization of the reaction mixture and formation of the white precipitate, cycloadduct was filtered off and crystallized from acetone—petroleum ether. **3β-Acetoxy-4**['], 5[']-**dihydro-5**[']β-**phenylpregn-5-eno[17α,16α-c]pyrazol-20-one (2a)** was synthesized from benzaldehyde tosylhydrazone sodium salt⁷ (1.1 g, 3.6 mmol) and DPA (1.0 g, 2.8 mmol). The yields of pyrazoline **2a** was 1.1 g (95%), crystalline compound, m.p. 174.5 °C (methanol—dichloromethane). ¹H NMR, δ: 0.85 (s, 3 H, 18-Me); 1.05 (s, 3 H, 19-Me); 2.05 (s, 3 H, acetate); 2.60 (s, 3 H, 21-Me); 2.95 (dt, 1 H, H(5')); 4.60 (m, 1 H, H(3)); 5.30 (m, 1 H, H(6)); 6.99 (dd, 2 H, Ph); 7.30 (m, 3 H, Ph). MS, m/z (I_{rel} (%)): 446 [M – N₂]⁺ (26); 403 [M – N₂, Ac]⁺ (13); 386 [M – CHPh]⁺ (14); 371 [M – CHPh, CH₃]⁺ (35); 343 [M – CHPh, CH₃, N₂]⁺ (78).

3β-Acetoxy-4['],5[']-**dihydro-5**[']β-(**4-fluorophenyl)pregn-5-eno-**[**17α**,**16α**-*c*]**pyrazol-20-one (2b)** was synthesized from 4-fluorobenzaldehyde tosylhydrazone sodium salt⁷ (0.52 g, 1.7 mmol) and DPA (0.37 g, 1.0 mmol). The yield of pyrazoline **2b** was 0.46 g (95%), colorless crystals, m.p. 166 °C (decomp.). ¹H NMR, δ: 0.88 (s, 3 H, 18-Me); 1.05 (s, 3 H, 19-Me); 2.05 (s, 3 H, acetate); 2.58 (s, 3 H, 21-Me); 2.95 (dt, 1 H, H(5')); 4.60 (m, 1 H, H(3)); 5.35 (m, 1 H, H(6)); 6.99 (m, 2 H, Ar); 7.15 (m, 2 H, Ar). MS, *m/z* (I_{rel} (%)): 464 [M – N₂]⁺ (36); 449 [M – N₂, Me]⁺ (7); 421 [M – N₂, Me, Ac]⁺ (5).

3β-Acetoxy-3 β-phenyl[16α,17α]cyclopropapregn-5-en-20one (3a). Pyrazoline **2a** (0.52 g, 1.1 mmol) was slowly heated at 180 °C and a pressure of 15 Torr. At melting temperature, foaming was observed. After gas evolution ceased and cooling of the reaction mixture, cyclopropane **3a** was obtained in the yield of 0.44 g (90%), m.p. 209–210 °C (from acetone—hexane). Found (%): C, 80.23; H, 8.56. $C_{30}H_{38}O_3$. Calculated (%): C, 80.68; H, 8.58. ¹H NMR, δ: 0.87 (s, 3 H, 18-Me); 1.08 (s, 3 H, 19-Me); 1.70 (s, 3 H, 21-Me); 2.05 (s, 3 H, acetate); 2.71 (dt, 1 H, H(5')); 4,62 (m, 1 H, H(3)); 5.40 (m, 1 H, H(6)); 7.05–7.30 (m, 5 H, Ph). MS, m/z (I_{rel} (%)): 446 [M]⁺ (20); 431 [M – CH₃]⁺ (0.1); 403 [M – Ac]⁺ (2.5); 386 [M – CHPh]⁺ (8).

3β-Acetoxy-3 'β-(4-fluorophenyl)[16α,17α]cyclopropapregn-5-en-20-one (3b) was obtained by pyrolysis of pyrazoline 2b (0.27 g, 0.57 mmol) in the yield of 0.253 g (96%), white powder. ¹H NMR, δ: 0.88 (s, 3 H, 18-Me); 1.02 (s, 3 H, 19-Me); 1.68 (s, 3 H, 21-Me); 2.03 (s, 3 H, acetate); 2.69 (dt, 1 H, H(5')); 4.62 (m, 1 H, H(3)); 5.40 (m, 1 H, H(6)); 6.85–7.10 (m, 4 H, Ar). MS, m/z (I_{rel} (%)): 464 [M]⁺ (55); 390 [M – Ac, Me]⁺ (25).

3´β-Phenyl[16α,17α]cyclopropapregn-4-ene-3,20-dione (1a). A suspension of acetate 3a (0.30 g, 0.67 mmol) in MeOH (55 mL) and KOH (86.5 mg, 1.54 mmol) in H₂O (0.5 mL) was stirred for 3 h at 40 °C. The reaction mixture was neutralized with 10% HCl, poured into ice-water (100 mL), and the colorless precipitate formed was filtered off. Chromatographically homogeneous 3β -hydroxy derivative (0.25 g, 92%) obtained after drying in air was subjected to Oppenauer oxidation by refluxing with Al(PrⁱO)₃ (325 mg) and cyclohexanone (4 mL) in toluene (30 mL) for 3 h. The reaction mixture was acidified with diluted AcOH, the organic layer was separated, washed to neutral, dried with anhydrous MgSO₄, and concentrated in vacuo. Purification of the oily residue by column chromatography (elution with petroleum ether—acetone, 96 : $4 \rightarrow 90$: 10) afforded conjugated ketone 1a in the yield of 0.20 g (80%), m.p. 249–251 °C (from diethyl ether-hexane). Found (%): C, 83.59; H, 8.59. C₂₈H₃₄O₂. Calculated (%): C, 83.54; H, 8.51. MS, $m/z (I_{rel} (\%): 402 [M]^+ (65))$, 387 $[M - 15]^+$ (10), 359 $[M - 43]^+$ (35). $[\alpha]_D$ +119.4 (c 1.30). ¹H NMR, δ: 0.96 (s, 3 H, Me(18)); 1.23 (s, 3 H, Me(19)); 1.75 (s, 3 H, Me(21)); 2.73 (d, 1 H, H(2'), J = 4.32 Hz); 5.74 (s, 1 H, H)H(4)); 7.05–7.30 (H_{arom}).

3 'β-(4-Fluorophenyl)[16α,17α]cyclopropapregn-4-ene-3,20dione (1b) was synthesized as described for 1a. From acetate 3b (0.25 g, 0.54 mmol), 3β-hydroxy derivative (0.205 g, 90%) was obtained, which was further subjected to Oppenauer oxidation to give conjugated ketone 1b in the yield of 0.166 g (82%), m.p. 211–213 °C (from diethyl ether—hexane). Found (%): C, 79.78; H, 8.14. C₂₈H₃₃FO₂. Calculated (%): C, 79.97; H, 7.91. MS, m/z (I_{rel} (%)): 420 [M]⁺ (53), 377 [M – 43]⁺ (30). [α]_D +121.6 (*c* 1.30). ¹H NMR, δ: 0.92 (s, 3 H, 18-Me); 1.20 (s, 3 H, 19-Me); 1.75 (s, 3 H, 21-Me); 2.72 (d, 1 H, H(2'), J = 4.32 Hz); 5.74 (s, 1 H, H(4)); 6.88–7.10 (H_{arom}).

[16 α ,17 α](Spiro[2.2]penta)pregn-4-ene-3,20-dione (5). 3 β -Acetoxy-4',5'-dihydro-3'-cyclopropapregn-5-eno[17 α ,16 α -c]pyrazol-20-one (m.p. 126–128 °C) synthesized by 1,3-dipolar cycloaddition according to the general procedure was subjected to pyrolysis at 180–200 °C. Column chromatography afforded 3 β -acetoxy[16 α ,17 α](spiro[2.2]penta)pregn-5-en-20-one acetate. Cleavage of the acetate group and subsequent oxidation as described above furnished compound 5, m.p. 148–149 °C (from hexane—acetone) (*cf.* Ref. 6: 142–145 °C). ¹H NMR, δ : 0.99 (s, 3 H, Me(18)); 1.19 (s, 3 H, Me(19)); 1.96 (s, 3 H, Me(21)); 5.72 (s, 1 H, H(4)).

Biological experiments. The ligand-receptor binding was estimated as earlier described.⁸ The experiments were carried out with sexually mature virgin female rats of a mixed population with the body mass of 200–250 g. For 4 days, animals daily received intramuscular injections of estradiol (10 μ g) in propylene glycol (200 μ L) and were decapitated on the 5th day. The uteri were minced and homogenized in a glass homogenizer for 5 min at 0–4 °C in a buffer solution containing 10 *mM* Tirs-HCl (pH 7.5), 10 *mM* KCl, 1 *mM* EDTA, 0.5 *mM* phenylmethylsulfonyl fluoride (PMSF), 1 *mM* dithiothreitol, 10% glycerol at a tissue—buffer ratio of 1 : 6. The homogenate was centrifuged (50000 g) for 1 h at 4 °C. Supernatant (cytosol) with protein concentration of 4–6 mg mL⁻¹ was used immediately .

Steroids. Progesterone and hydrocortisone (Sigma, USA), $[1,2,6,7^{-3}H]$ progesterone with a specific radioactivity of 86 Ci mmol⁻¹ (St. Petersburg) were used.

Relative binding affinity (RBA) of the steroids for PR were measured using [³H]progesterone in the presence of hydrocortisone (3 μ mol L⁻¹), which was added to block possible interaction of [³H]progesterone with blood transcortin. Uterine cytosol (100 μ L) was incubated for 20 h at 0–4 °C with a steroid mixture in the buffer (100 μ L) containing [³H]-labeled ligand $((60-80)\cdot 10^3 \text{ dpm}, \text{ final concentration of } 3-6 \text{ nmol } L^{-1})$ and unlabeled competitor (final concentrations from 0 to 10 μ mol L⁻¹). The free and protein-bound ligands were separated by incubation with 2% suspension (100 µL) of Norit A (Serva, Germany) coated with Dextrane-70 (Fluka, Switzerland) for 5 min at 0-4 °C. After centrifugation for 5 min at 3000 g, the aliquots (250 mL) were taken and radioactivity was measured. The incubation was carried out in quartz tubes coated with BSA. Radioactivity were measured using dioxane scintillator at the counting efficiency of 20%. The protein content was determined using Coumassie blue.¹⁰ From 3 to 4 independent experiments were performed in duplicate. The equilibrium dissociation constants (K_d) were determined by adjustment of the K_d and B_{max} parameters providing the minimum deviation of the experimental data from "one protein-two ligands" kinetic model. The dimensionless RBA values were calculated as a ratio of the K_d values for progesterone and the ligand under study obtained in the independent experiments, the calculated RBA values were averaged.

 Table 2. Crystallographic characteristics, details of X-ray experiment and refinement statistics for compound 1a

Parameter	Value	
Molecular formula	C ₂₈ H ₃₄ O ₂	
Molecular weight	402.55	
T/K	120(2)	
λ/Å	0.71073	
Crystal system	Monoclinic	
Space group	P21	
a/Å	9.1768(13)	
b/Å	6.6126(9)	
c/Å	17.943(3)	
α/deg	90	
β/deg	96.500(3)	
γ/deg	90	
$V/Å^3$	1081.8(3)	
Z	2	
$d_{\rm calc}/{\rm g}{\rm cm}^{-3}$	1.236	
Absorption coefficient, μ/mm^{-1}	0.075	
<i>F</i> (000)	436	
Crystal size/mm	$0.55 \times 0.40 \times 0.25$	
Scanning range θ/deg	2.23-27.00	
hkl Ranges	$-11 \le h \le 11, -8 \le k \le 8,$	
	$-22 \le l \le 22$	
Number of reflections measured	10296	
reflections independent reflections	2540	
R _{int}	0.0278	
Number of refined parameters	271	
Number of reflections with $I \ge 2\sigma(I)$	2242	
R_1, wR_2	0.0400, 0.0898	
R Factors on all reflections		
R_1, wR_2	0.0459, 0.0931	
GOOF	1.021	
Residual electron density max/min, e Å ⁻³	0.191/-0.161	

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