

Synthesis of progesterone heterocyclic derivatives of potential antimicrobial activity

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The aim of this work was to synthesize steroidal heterocycles and to elucidate the potential role of these compounds as antimicrobial agents. The synthesis of steroidal heterocycles containing the pyrazole, isoxazole, thiazole, pyrane, pyridine, pyridazine, or benzopyrane ring attached to the pregnene nucleus is reported. Progesterone (1) reacts with dimethyl formamide dimethyl acetal to form enamine 2. Heterocyclization of 2 with hydrazines, hydroxylamine, glycine, ethyl acetoacetate or cyanomethylene afforded novel steroidal heterocyclic derivatives. The *in vitro* antimicrobial evaluation showed that all synthesized compounds show activity against the used strains of Gram positive bacteria and fungi.

Keywords: progesterone, enamine, pyrazole, pyrane, antimicrobial

Progesterone is one of the most important hormones of the steroidal pregnane series, secreted by corpus luteum and by placenta, which can be regarded as a hormonal balancer, particularly of estrogens. It also helps to create a balance of all other steroids and has intrinsic calming and diuretic properties. It is important in women and its importance in men for the maintenance of prostate health is appreciated. Moreover, progesterone has a neuroprotective effect (1, 2). Thus, due to the importance of progesterones to the human body and in continuation of our previous work on heterocyclic synthesis (3–5), we would like to report here on the formation and characterization of new promising steroidal heterocyclic derivatives derived from progesterone. Detection of many emerging chemicals of concern, including antimicrobials and steroid hormones, in the environment was increased in the past decade with the advancement of analytical techniques. All compounds produced in this work are novel and their synthetic pathways are also novel. Therefore, the *in vitro* antimicrobial activity of the new steroidal derivatives should be tested against Gram positive and negative bacteria, and fungi.

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EXPERIMENTAL

Synthetic methods, analytical and spectral data

Starting steroids were purchased from Sigma Company (Germany). Appropriate precautions in handling moisture sensitive compounds were undertaken. Melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. Elemental analyses were performed on a Yanaco CHN Corder elemental analyzer (Japan). IR spectra (ν , cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer (Shimadzu, Japan). ^1H NMR spectra were obtained on a Jeol 300 MHz (Japan) spectrometer in DMSO-d_6 as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

For the nomenclature of steroid derivatives, we used the definitive rules for the nomenclature of steroid published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC (6, 7).

Compound **2** was synthesized by reacting progesterone with dimethylformamide dimethylacetal in refluxing xylene solution according to the literature procedure (8). Synthetic pathways are presented in Schemes 1, 2, 3 and 4, and physicochemical and spectral data for the synthesized compounds are given in Tables I and II.

Synthesis of 17-(pyrazol-3-yl)androst-4-en-3-one (3a) and 17-(1-phenylpyrazol-3-yl)androst-4-en-3-one (3b)

General procedure. – To a solution of compound **2** (0.66 g, 0.002 mol) in absolute ethanol (30 mL), either hydrazine hydrate (0.1 g, 0.002 mol) or phenylhydrazine (0.2 g, 0.002 mol) was added. The reaction mixture, in each case, was heated under reflux for 5 h, then left to cool at room temperature and poured into ice/water mixture. The formed solid product collected by filtration was dried and crystallized to give either **3a** (crystallized from ethanol) or **3b** (crystallized from 1,4-dioxane).

Synthesis of 17-(isoxazol-6-yl)androst-4-en-3-one (4)

To a solution of compound **2** (0.66 g, 0.002 mol) in absolute ethanol (30 mL), containing sodium acetate (0.16 g, 0.002 mol), an equivalent amount of hydroxylamine hydrochloride (0.13 g, 0.002 mol) in water (5 mL) was added. The reaction mixture was boiled under reflux for 3 h then left to cool to 20 °C and poured over ice/water mixture. The formed solid product was collected by filtration and crystallized from 1,4-dioxane.

Synthesis of 17-(2-amino-3-cyanopyran-6-yl)androst-4-en-3-one (6a)

Malononitrile (0.13 g, 0.002 mol) was added to a suspension of compound **2** (0.66, 0.002 mol) in sodium ethoxide (0.01 mol) [prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (5 mL)]. The reaction mixture was heated under reflux for 5 h, then left to cool at room temperature, poured into ice/water mixture and neutralized with dilute hydrochloric acid. The solid product **6a**, which was formed through the formation of the non isolable intermediate **5a**, was collected by filtration, dried and crystallized from ethanol.

Table I. Physico-chemical data for synthesized compounds

Compd. No.	Yield (%)	M.p. (°C)	Mol. formula (M _r)	Found/calcd. (%)			
				C	H	N	S
3a	75	100–102	C ₂₂ H ₃₀ N ₂ O (338.49)	77.32	8.74	8.12	–
				77.06	8.93	8.27	–
3b	69	97–99	C ₂₈ H ₃₄ N ₂ O (414.59)	80.94	8.13	6.55	–
				81.11	8.26	6.75	–
4	74	165–167	C ₂₂ H ₂₉ NO ₂ (339.48)	77.67	8.33	4.03	–
				77.83	8.61	4.12	–
6a	69	152–155	C ₂₅ H ₃₂ N ₂ O ₂ (392.54)	74.33	8.11	7.08	–
				74.49	8.21	7.13	–
6b	68	100–101	C ₂₅ H ₃₁ NO ₃ (393.53)	76.22	7.75	3.32	–
				76.30	7.94	3.55	–
7	72	118–120	C ₃₀ H ₄₃ NO ₄ (481.68)	74.71	8.65	2.82	–
				74.80	8.99	2.90	–
9	76	178–179	C ₂₆ H ₃₃ O ₄ (410.55)	76.00	8.31	–	–
				76.06	8.34	–	–
11	78	109–110	C ₂₅ H ₃₁ ON ₃ (389.54)	77.00	7.98	10.56	–
				77.08	8.02	10.78	–
13	68	188–189	C ₂₃ H ₃₁ NO (337.51)	81.64	9.12	4.01	–
				81.85	9.25	4.15	–
14	68	188–190	C ₂₄ H ₃₃ N ₃ O ₂ (395.55)	72.62	8.25	10.53	–
				72.87	8.40	10.62	–
15	77	70–72	C ₃₁ H ₃₇ N ₃ O ₂ (483.65)	76.76	7.58	8.54	–
				76.98	7.71	8.68	–
17	66	210–212	C ₂₇ H ₃₄ N ₄ O ₃ (462.59) (462)	69.82	7.12	12.31	–
				70.10	7.40	12.11	–
18	70	210–212	C ₂₈ H ₃₈ N ₂ O ₂ (434.62)	77.52	8.63	6.24	–
				77.37	8.81	6.44	–
19	75	167–168	C ₂₆ H ₃₅ N ₃ O ₃ S (469.65)	69.67	7.35	8.74	6.82
				69.94	7.51	8.94	6.82
20	75	84–86	C ₃₁ H ₃₆ N ₂ O ₄ (500.64)	74.17	7.04	5.39	–
				74.37	7.24	5.59	–

Synthesis of 17-(2-hydroxy-3-cyanopyran-6-yl)androst-4-en-3-one (6b)

To a solution of compound **2** (0.66, 0.002 mol) in ethanol (30 mL), containing a catalytic amount of triethylamine (0.5 mL), an equivalent amount of ethyl cyanoacetate (0.23 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 5 h; after cooling at room temperature, it was poured into ice/water mixture and neutralized with di-

Table II. Spectral data of newly synthesized products

Compd. No.	IR (ν , cm^{-1})	^1H NMR (δ , ppm) (DMSO- d_6)	MS (M^+)
3a	3300 (NH), 2980, 2860 (CH ₃ , CH ₂), 1715 (C=O), 1659(C=N), 1576 (C=C)	0.75 (s, 3H, 18-CH ₃), 0.92 (s, 3H, 19-CH ₃), 4.97 (s, 1H, C4-H), 6.14 (d, 1H, pyrazole, H-4), 6.22 (d, 1H, pyrazole, H-5), 9.11 (s, 1H, NH, D ₂ O-exchangeable)	338
3b	3050 (CH-aromatic), 2980, 2863 (CH ₃ , CH ₂), 1715 (C=O), 1650 (C=N), 1590 (C=C)	0.75 (s, 3H, 18-CH ₃), 0.92 (s, 3H, 19-CH ₃), 4.82 (s, 1H, C4-H), 6.19 (d, 1H, pyrazole, H-4), 6.34 (d, 1H, pyrazole H-5), 7.31–7.56 (m, 5H, C ₆ H ₅)	414
4	2987, 2855 (CH ₃ , CH ₂), 1718 (C=O), 1650 (C=N), 1580 (C=C)	0.72 (s, 3H, 18-CH ₃), 0.89 (s, 3H, 19-CH ₃), 4.70 (s, 1H, C4-H), 4.83 (d, 1H, isoxazole, H-4), 5.23 (d, 1H, isoxazole, H-5)	339
6a	3320 (NH ₂), 2980, 2860 (CH ₃ , CH ₂), 2225, (CN), 1710 (C=O), 1580 (C=C)	0.88 (s, 3H, 18-CH ₃), 1.05 (s, 3H, 19-CH ₃), 4.72 (s, 1H, C4-H), 6.33 (s, 1H, NH ₂ , D ₂ O exchangeable), 7.03 (d, 1H, pyrane, H-4), 7.12 (d, 1H, pyrane, H-5)	392
6b	3400 (OH), 2982, 2820 (CH ₃ , CH ₂), 1715 (C=O), 1590 (C=C)	0.83 (s,3H, 18-CH ₃), 1.07 (s, 3H, 19-CH ₃), 4.76 (s, 1H, C4-H), 7.03 (d, 1H, pyrane, H-4), 7.12 (d, 1H, pyrane, H-5), 9.57 (s, 1H, OH, D ₂ O exchangeable)	393
7	2980, 2863 (CH ₃ , CH ₂), 1735, 1722, 1710 (3C=O), 1590 (C=C)	0.83 (s, 3H, 18-CH ₃), 1.03 (s, 3H, 19-CH ₃), 1.32 (t, 3H, ester CH ₃), 2.34 (s, 1H, COCH ₃), 4.22 (q, 2 H, ester CH ₂), 4.82 (s, 1H, C4-H), 4.95 (d, 1H, CH), 5.23 (d, 1H, CH). 4.95 (d, 1H, CH), 5.23 (d, 1H, CH)	481
9	3300 (OH), 2980, 2863 (CH ₃ , CH ₂), 1725, 1708 (2C=O), 1590 (C=C)	0.79 (s, 3H, 18-CH ₃), 0.95 (s, 3H, 19-CH ₃), 2.23 (s, 3H, COCH ₃), 6.91 (d, 1H, pyran, H-4), 7.02 (d, 1H, pyran, H-5), 9.57 (s, 1H, OH, D ₂ O-exchangeable)	410
11	2980, 2860 (CH ₃ , CH ₂), 2225 (CN), 1710 (C=O), 1580 (C=C)	0.78 (s, 3H, 18-CH ₃), 0.95 (s, 3H, 19-CH ₃), 4.70 (s, 1H, C4-H), 1.25, 1.32 (2s, 6H, 2 CH ₃), 2.11 (s, 1H, CH ₂), 5.93 (d, 1H, pyradizine, CH), 6.03 (d, 1H, pyradizine, CH)	389
13	3334 (NH), 2980, 2860 (CH ₃ , CH ₂), 1715 (C=O), 1576 (C=C)	0.75 (s, 3H, 18-CH ₃), 0.92 (s, 3H, 19-CH ₃), 4.97 (s, 1H, C4-H), 5.23 (m, 2H, pyrol, H-2, H-3), 8.14 (s, 1H, pyrol, CH), 9.11 (s, 1H, NH, D ₂ O – exchangeable)	337
14	3360 (NH), 2980, 2855 (CH ₃ , CH ₂), 2225 (CN), 1718, 1715 (2C=O), 1630 (C=N), 1580 (C=C)	0.78 (s, 3H, 18-CH ₃), 0.95 (s, 3H, 19-CH ₃), 1.15 (s, 3H, 21-CH ₃), 2.11 (s, 2H, CH ₂), 4.83 (s, 1H, C4-H), 9.55 (s, 1H, NH, D ₂ O exchangeable)	395

15	3320 (NH), 3030 (CH-aromatic), 2982, 2868 (CH ₃ , CH ₂), 2225 (CN), 1730, 1710(2C=O), 1640 (C=N), 1590 (C=C)	0.75 (s, 3H, 18-CH ₃), 0.97(s, 3H, 19-CH ₃), 1.10 (s, 3H, 21-CH ₃), 4.73 (s, 1H, C4-H), 5 H, C ₆ H ₅), 8.51 (s, 1H, NH, D ₂ O exchangeable)	483
17	3435 (NH ₂), 2982, 2868 (CH ₃ , CH ₂), 2220 (CN), 1730, 1718, 1710 (3C=O), 1640 (C=N), 1590 (C=C)	0.75 (s, 3H, 18-CH ₃), 0.97 (s, 3H, 19-CH ₃), 1.10 (s, 3H, 21-CH ₃), 4.31 (s, 2H, pyridine, H-5), 4.91 (s, 1H, C4-H), 6.33 (s, 2H, NH ₂ , D ₂ O exchangeable)	462
18	2980, 2860 (CH ₃ , CH ₂), 1725, 1710 (2C=O), 1640 (C=N), 1580 (C=C)	0.78 (s, 3H, 18-CH ₃), 0.95(s, 3H, 19-CH ₃), 1.05 (s, 3H, 21-CH ₃), 1.21, 1.34 (2s, 6H, 2CH ₃), 4.70 (s, 1H, C4-H), 6.28, 6.43 (2s, 2H, pyridine, H-3, pyridine H-5)	434
19	3325 (NH), 2980, 2860 (CH ₃ , CH ₂), 1718, 1710, 1695 (3C=O), 1640 (C=N)	0.78 (s, 3H, 18-CH ₃), 0.95 (s, 3H, 19-CH ₃), 4.93 (s, 2H, CH ₂), 5.72(s, 2H, thiazole, H-5), 8.25 (s, 1H, NH, D ₂ O exchangeable)	469
20	3325 (NH), 3050 (CH aromatic), 2980, 2855 CH ₃ , CH ₂), 1730, 1710, 1690 (3 C=O), 1630 (C=N), 1578 (C=C)	0.78 (s, 3H, 18-CH ₃), 0.95 (s, 3H, 19-CH ₃), 1.05 (s, 3H, 21-CH ₃), 4.67 (s, 1H, C4-H), 6.99 (s, 1H, coumarin H-4), 7.32-7.55 (m, 4 H, C ₆ H ₄), 11.2 (s, 1H, NH, D ₂ O exchangeable)	500

lute hydrochloric acid. The formed solid product of **6b**, which was formed through the intermediate formation of the non isolable **5b**, was filtered off, dried, and crystallized from ethanol.

Synthesis of 21-(dimethylaminoacryloyl)-20-(ethylacetoacetato)pregn-4-en-3-one (7)

To a solution of compound **2** (0.66 g, 0.002 mol) in ethanol containing a catalytic amount of ammonium acetate (0.1 g), an equivalent amount of ethyl acetoacetate (0.23 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 4 h, then left to cool at room temperature and poured into ice/water mixture. The formed solid product was filtered off, dried and crystallized from 1,4-dioxane.

Synthesis of 17-(2-hydroxy-3-acetylpyran-6-yl)androst-4-en-3-one (9)

To a solution of **2** (0.66 g, 0.002 mol) in toluene (30 mL), ethyl acetoacetate (0.23 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 5 h and then the reaction mixture was concentrated under vacuum. The solid product **9**, was formed through the intermediate formation of the non isolable **8**; it was dried and crystallized from 1,4-dioxane.

Synthesis of 17-(3-cyanomethylpyridazin-6-yl)androst-4-en-3-one (11)

To a solution of compound **2** (0.66 g, 0.002 mol) in 1,4-dioxane (30 mL), an equivalent amount of freshly prepared cyanoacetic acid hydrazide (0.19 g, 0.002 mol) [prepared by the addition of an equivalent amount of hydrazine hydrate to ethyl cyanoacetate under stirring at room temperature] was added; the reaction mixture was heated under reflux for 6 h and then concentrated under vacuum. The solid product of **11**, which was for-

med *via* the intermediate formation of the non isolable **10**, was filtered off, dried and crystallized from ethanol.

Synthesis of 17-(pyrol-3-yl)androst-4-en-3-one (13)

To a solution of compound **2** (0.66 g, 0.002 mol) in acetic anhydride (30 mL), an equivalent amount of glycine (0.15 g, 0.002 mol) was added, the reaction mixture was heated under reflux for 3 h, and then concentrated under vacuum. The solid product of **13**, which was formed *via* the intermediate formation of the non isolable **12**, was filtered off, dried and crystallized from ethanol.

Synthesis of 20-(cyanoacetylhydrazono)pregn-4-en-3-one (14)

A dry mixture of progesterone **1** (1.57 g, 0.005 mol) and freshly prepared cyanoacetic acid hydrazide (0.49 g, 0.005 mol) was heated in an oil bath at 130 °C for 1 h and then acetic acid (5 mL) was added. The solid product formed upon cooling at room temperature was triturated with ethanol, collected by filtration and crystallized from 1,4-dioxane.

Synthesis of 20-(1-phenyl-2-cyanopropenoylhydrazono)pregn-4-en-3-one (15)

To a solution of **14** (0.79 g, 0.002 mol) in absolute ethanol (30 mL) containing a catalytic amount of pyridine (0.5 mL), benzaldehyde (0.21 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 4 h and then cooled at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product was filtered off, dried and crystallized from methanol.

Synthesis of 20-hydrazono-N-(4-amino-3-cyano-2,6-dioxopyridin-1-yl)pregn-4-en-3-one (17)

To a solution of compound **14** (0.79 g, 0.002 mol) in ethanol containing a catalytic amount of pyridine (0.5 mL), an equivalent amount of ethyl cyanoacetate (0.22 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 4 h, then left to cool at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product of **17**, which was formed *via* the intermediate formation of the non isolable **16**, was filtered off, dried and crystallized from methanol.

Synthesis of 20-hydrazono-N-(4,6-dimethyl-2-oxopyridin-1-yl)pregn-4-en-3-one (18)

To a solution of **14** (0.73 g, 0.002 mol) in acetic anhydride (30 mL), an equivalent amount of acetyl acetone (0.2 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 3 h and then concentrated under vacuum; the solid product so formed was filtered off, dried and crystallized from ethanol.

Synthesis of 20-(4-oxothiazol-2-acetylhydrazonyl-2-yl)pregn-4-en-3-one (19)

To a solution of compound **14** (0.79 g, 0.002 mol) in ethanol containing a catalytic amount of pyridine (0.5 mL), an equivalent amount of thioglycolic acid (0.18 mL, 0.002 mol) was added. The reaction mixture was heated under reflux for 5 h then left to cool at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product was filtered off, dried and crystallized from ethanol.

Synthesis of 20-(coumarin-3-carbohydrazonyl)pregn-4-en-3-one (20)

To a solution of **14** (0.79 g, 0.002 mol) in dimethylformamide (30 mL) containing a catalytic amount of piperidine (0.5 mL), salicylaldehyde (0.002 g, 0.002 mol) was added, the reaction mixture was heated under reflux for 5 h, cooled at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product was filtered off, dried and crystallized from methanol.

Analytical and spectral data of all products are in agreement with the proposed structure.

Antimicrobial activity

The *in vitro* antimicrobial activity of the structurally promising steroidal heterocyclic derivatives **2**, **3a,b**, **6b**, **7**, **9**, **11**, **13**, **15**, **14**, **17**, **18** and **20** against two strains of Gram positive bacteria (*Bacillus subtilis* CECT 498 and *Bacillus cereus* CECT 148), two strains of Gram negative bacteria (*Escherichia coli* ECT 101 and *Pseudomonas aeruginosa*) and *Candida albicans* CECT 1394 as a representative species of fungi was investigated. The newly synthesized products were dissolved in aqueous ethanol to give a logarithmic series of concentrations from 2 to 256 mg L⁻¹ upon tenfold dilution with the growth medium and spore suspension of the test fungi. The toxicity of compounds was determined *via* pipette additions into the wells of multi-well slides, followed by 25 µL of culture medium. The inoculated slides were then incubated at 25 °C until short germ tubes appeared; approximately 50 µm length (at 0 h) was measured. Five-µL volumes of the prepared compound test solutions were added to the inoculated wells, one control well on each slide being treated with solvent only. The slides were then returned to the incubator until

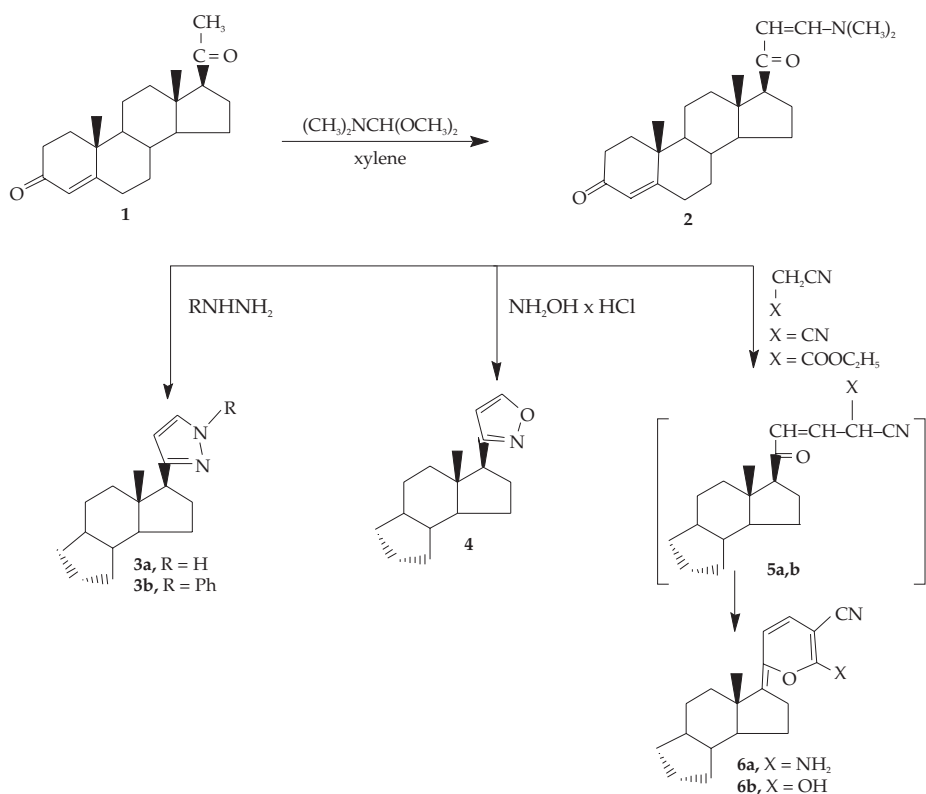
Table III. Antimicrobial activities of tested compounds

Compd.	MIC (µg mL ⁻¹) (zone of inhibition, mm)			
	<i>E. coli</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>C. albicans</i>
2	12.50 (6)	20.52 (8)	6.25 (4)	8.65 (4)
3a	–	6.25 (15)	20.01 (8)	30.23 (6)
3b	–	12.32 (3)	16.32 (8)	14.40 (4)
6b	–	12.30 (4)	4.22 (6)	12.55 (12)
7	–	25.36 (8)	23.63 (6)	26.12 (3)
9	–	20.15 (4)	23.16 (9)	100.00 (5)
11	16.64	0.06 (2)	6.33 (5)	50.17 (11)
13	–	18.32 (5)	6.22 (2)	0.40 (10)
15	10.46 (4)	8.66 (6)	25.33 (5)	12.22 (8)
17	–	6.05 (6)	12.42 (2)	4.55 (10)
19	–	0.05 (9)	3.13 (10)	0.61 (6)
20	–	12.34 (7)	6.13 (4)	0.40 (5)
Ampicillin	6.25	3.13	12.50	(10)
–				
Cycloheximide	–	–	–	12.50 (6)

germ tubes ($400 \pm 50 \mu\text{m}$) long were visible in the control wells. Further growth was arrested by the addition of lactophenol aniline blue to each of the wells. The minimal inhibitory concentration (*MIC*, in $\mu\text{g mL}^{-1}$) was determined using an adaptation of the agar streak dilution method based on radial diffusion (9, 10). Under the same conditions, ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. *MIC* was considered to be the lowest concentration of the tested compound (in dimethylformamide) that inhibits the growth of bacteria or fungi in the plate (Table III). Diameters of the inhibition zones corresponding to *MICs* are also presented in Table III.

RESULTS AND DISCUSSION

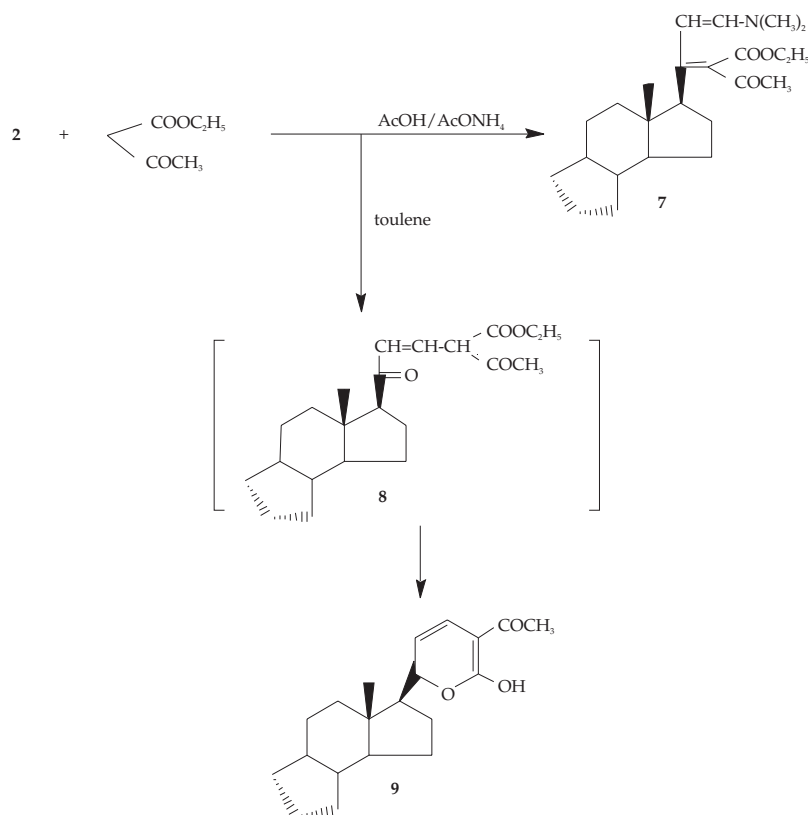
In this work, the reaction of progesterone (1) with dimethyl formamide dimethylacetal was studied. The reaction took place in xylene to give the enamine derivative 2 (8) (Scheme 1).



Scheme 1

The heterocyclization of compound **2** with different chemical reagents was studied with the aim of forming steroidal heterocyclic compounds with potential biological activities. Thus, compound **2** reacted with either hydrazine hydrate or phenylhydrazine to give the pyrazolylandrostenone derivatives **3a** and **3b**, respectively, and the reaction of compound **2** with hydroxylamine hydrochloride gave the isoxazolyandrostenone derivative **4**. The ^1H NMR of the latter product showed the presence of two doublets at δ 4.83 and 5.23 ppm for the isoxazole protons at C-4 and C-5, respectively. The reaction of compound **3** with either malononitrile or ethyl cyanoacetate gave the corresponding amino pyranilyandrostenone derivatives **6a,b**. Formation of the latter product took place through the intermediate formation of **5a,b** (Scheme 1).

The reaction of compound **2** with ethyl acetoacetate in acetic acid afforded the Knoevenagel condensation product **7**. On the other hand, carrying the same reaction in refluxing toluene gave the 3-acetyl-2-hydroxy pyranilyandrostenone derivative **9**. The reaction took place through the intermediate formation of **8** (Scheme 2). The reaction of compound **2** with cyanoacetylhydrazone in 1,4-dioxane gave pyridazinylandrostenone



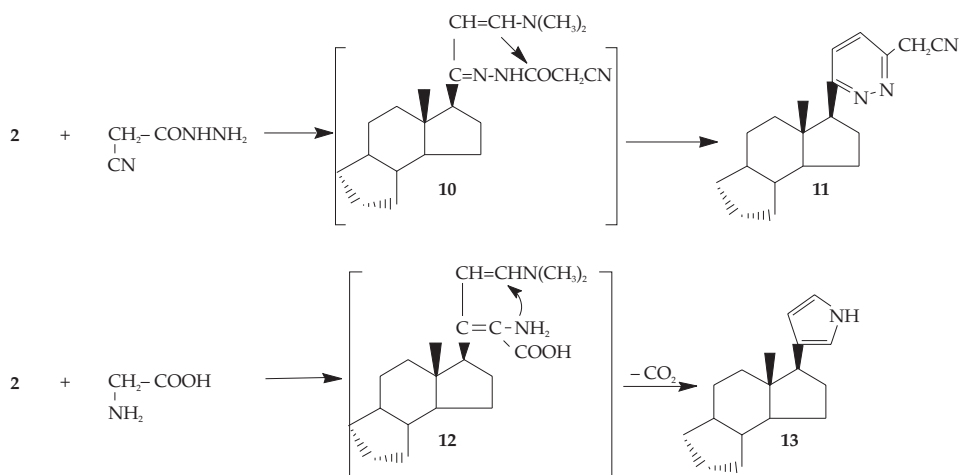
Scheme 2

derivative **11** *via* the intermediate formation of **10** (Scheme 3). Thus, IR spectrum of compound **11** showed the presence of one CN group stretching at ν 2225 cm^{-1} . Moreover, the ^1H NMR spectrum showed the presence of two singlets at δ 0.78 and 0.95 ppm corresponding to two CH_3 groups, a singlet at δ 2.11 ppm for the CH_2 group, a singlet at δ 4.70 ppm for C4-H and two doublets at δ 5.93 and 6.03 ppm corresponding to the pyridazine H-4 and H-5.

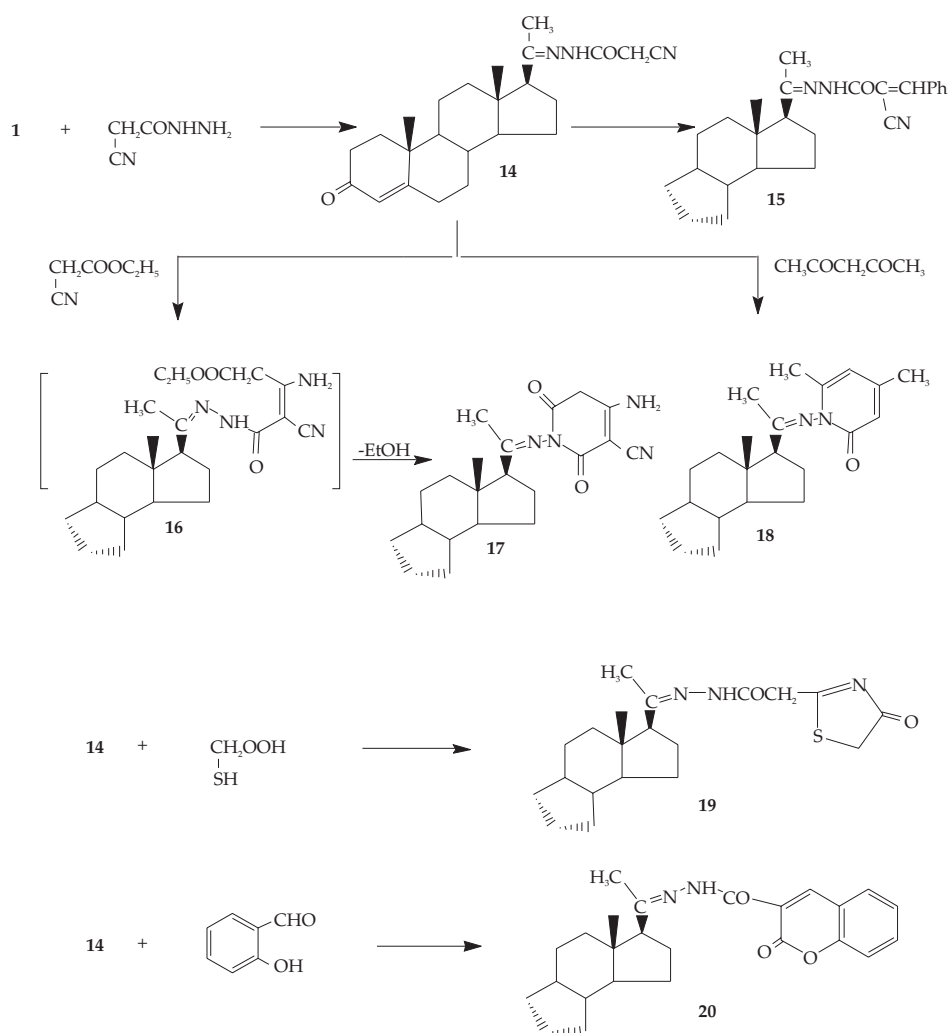
Compound **2** showed interesting reactivity when reacting with amino acids. Thus, with glycine in acetic anhydride it gave the pyrolylandrostenone derivative **13**. Formation of **13** can be explained in terms of the first condensation of the glycine CH_2 group with C=O group at C-20 of compound **2** to afford the intermediate **12** followed by cyclization and decarboxylation (Scheme 3). The analytical and spectral data of compound **13** are in agreement with the proposed structure (Tables I and II). Thus, the IR spectrum of compound **13** showed an NH stretching at ν 3334 cm^{-1} . Moreover, the ^1H NMR spectrum revealed the presence of two singlets at δ 0.75 and 0.92 ppm corresponding to the two CH_3 groups at C-18, C19, a singlet at δ 4.97 ppm for pyrazol H-4, a multiplet at δ 5.23 ppm for pyrol H-2, H-3 and a siglet at δ 9.11 ppm corresponding to the NH group.

The reaction of progesterone (**1**) with cyanoacetic acid hydrazide, in refluxing 1,4-dioxane gave the cyanoacetylhydrazonopregnene derivative **14**. The condensation of C=O group of progesterone (**1**) with the hydrazide to give hydrazone proceeds in parallel with another reaction recently reported by our group (11) (Scheme 4). The IR spectrum of the latter product showed a CN stretching at ν 2225 cm^{-1} and two C=O groups stretching at ν 1718 and 1715 cm^{-1} . Moreover, the ^1H NMR showed the presence of a singlet at δ 2.11 ppm for the CH_2 group (D_2O -exchangeable), a singlet at δ 9.55 ppm for a NH group. The MS spectrum of **14** showed $m/z = 395$ (M^+).

Compound **14** reacted with benzaldehyde to give the benzal derivative **15**. The reaction of **14** with ethyl cyanoacetate gave **17**. Formation of the latter product took place *via* the intermediate formation of **16**. On the other hand, compound **14** reacted with acetyl-



SHEMA 3



SHEMA 4

acetone to give the oxopyridinyl pregnene derivative **18** (Scheme 4). The reaction of compound **14** with thioglycolic acid gave the thiazolyl pregnene derivative **19** (12). The structure of the latter compound was based on analytical and spectral data (Tables I and II). Thus, the IR spectrum showed an NH stretching at ν 3325 cm^{-1} beside three C=O groups stretching at ν 1718, 1710 and 1695 cm^{-1} and the ^1H NMR spectrum of **19** which showed two singlets at δ 0.78, 0.95 ppm for the two CH_3 groups, a singlet at δ 4.93 for the CH_2 , a singlet at δ 5.72 ppm for the thiophene H-2 and a singlet at δ 8.25 ppm corresponding to the NH group. The MS spectrum of **19** showed $m/z = 469$ (M^+). On the other

hand, the reaction of **14** with salicylaldehyde gave the coumarin derivative **20** (Scheme 4). Formation of coumarin *via* the reaction of salicylaldehyde with cyanomethylene reagents has been reported in literature (13, 14) where the condensation of salicylaldehyde with the active methylene of the cyanomethylene reagent to form the corresponding non isolable arylidene was followed by cyclization and ammonia liberation to give the isolable coumarin product.

From Table III, it is obvious that no compound showed activity against *Pseudomonas aeruginosa* starting from DMSO solutions of 1000 $\mu\text{g mL}^{-1}$ of each compound. Compounds **2** (dimethylaminoacryloyl pregnenone), **11** [(17-(3-cyanomethylpyridazin-6-yl)-androst-4-en-3-one)] and **15** [20-(1-phenyl-2-cyanopropenylhydrazono)pregn-4-en-3-one] were active against *Escherichia coli* only. The results in Table III also clearly revealed that all tested compounds in the present study, except for compounds **11** and **19** [20-(4-oxothiazol-2-acetylhydrazonyl-2-yl)pregn-4-en-3-one], were active against Gram positive bacteria. However, compound **19** showed the least activity of the tested compounds. Compounds **2**, **11**, **15** are even more potent than the reference drug, ampicillin. Also, compounds **2**, **3a** [17-(pyrazol-3-yl)androst-4-en-3-one], **3b** [17-(1-phenylpyrazol-3-yl)-androst-4-en-3-one], **6b** [17-(2-hydroxy-3-cyanopyran-6-yl)androst-4-en-3-one], **7** [21-(dimethylaminoacryloyl)-20-(ethylacetoacetato)pregn-4-en-3-one], **9**, **11** and **15** are the most active compound against *Candida albicans*. Pyranylandrostenone derivative **9** **6b** [17-(2-hydroxy-3-acetylpyran-6-yl)androst-4-en-3-one] showed the maximum activity against *C. albicans*. Compounds **7** (with acetyl and ethyl ester groups) and **15** showed activity against *C. albicans* very close to that of the reference cycloheximide and higher than compounds **19** and **20** [20-(coumarin-3-carbohydrazonyl)pregn-4-en-3-one]. Compounds **2**, **6b**, **11**, **13**, **19** and **20** showed lower activity against *B. subtilis* than the reference ampicillin. One can notice the difference between the structures of compounds **2** and **7**; condensing the C=O group in **2** with the acetyl and ethyl ester methino groups to produce compound **7** increases the activity of **7** against *Candida albicans*. The coumarin derivative **20** showed higher activity towards *E. coli* than the thiazole derivative **19**.

CONCLUSIONS

The structure activity relationship suggested that heterocyclization reactions for enamine derivative of progesterone into pyrazole, isoxazole, pyrrol, thiazole and coumarin derivatives resulted in products with high antibacterial and antifungal activity. These findings encourage us to explore new molecules by introducing potent moieties, like heterocyclic ring systems described in this work, into other hormones. Our prediction is that these compounds with new ring systems may show even better antimicrobial activity.

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S A Ž E T A K

Sinteza heterocikličkih derivata progesterona s antimikrobnim djelovanjem

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U radu je opisana sinteza steroidnih heterocikličkih spojeva i evaluacija njihovog antimikrobnog djelovanja. Sintetizirani spojevi sadrže pirazol, izoksazol, tiazol, piran, piridin, piridazin ili benzopiran na pregnenskoj jezgri. Progesteron (**1**) je prvo u reakciji s dimetil formamid dimetil acetalom dao enamin **2**. Novi steroidni heterociklički derivati dobiveni su heterociklizacijom spoja **2** s hidrazinima, hidroksilaminom, glicinom, etil-acetoacetatom i cijanometilenom. Antimikrobno vrednovanje *in vitro* pokazalo je da su svi sintetizirani spojevi aktivni protiv testiranih Gram pozitivnih bakterija i gljivica.

Ključne riječi: progesteron, enamin, pirazol, piran, antimikrobno sredstvo

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