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SYNTHESIS AND ANTITUBERCULOSIS ACTIVITY OF SEVERAL STEROIDS FROM 3β-ACETOXY-5α-PREGN-16-EN-20-ONE

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The semicarbazone and isonicotinoylhydrazone of 5α-pregn-2-en-20-one, which was prepared from 3β-acetoxy-5α-pregn-16-en-20-one, were synthesized for the first time. The antituberculosis activity of these and semicarbazones and isonicotinoylhydrazones of saturated, unsaturated, and adamantane-modified ketosteroids synthesized by us earlier was studied in vitro *experiments.*

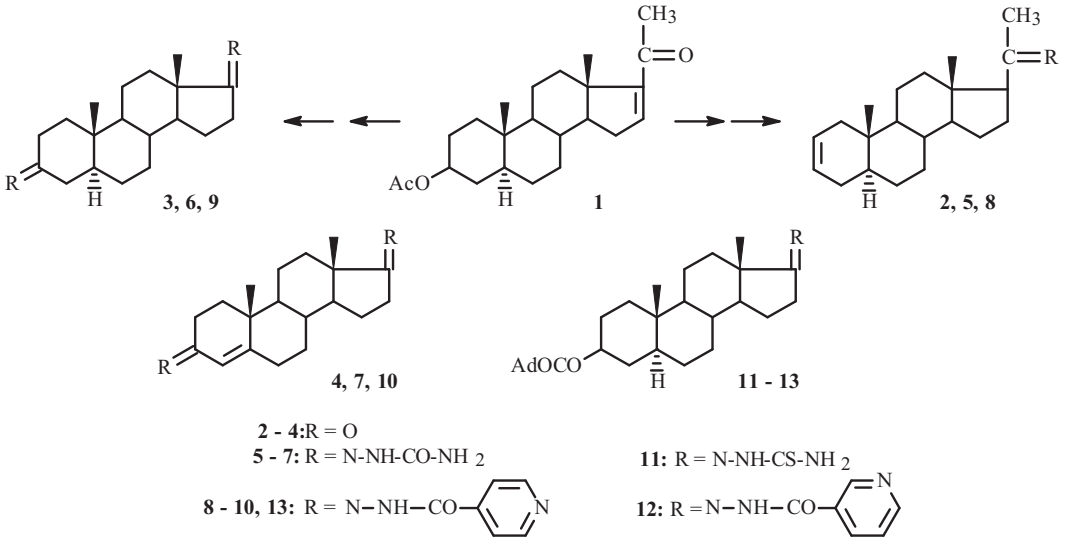
Keywords: ketosteroids, semicarbazones, isonicotinoylhydrazones, antituberculosis activity.

The development of resistance in *Mycobacterium tuberculosis* to drugs for treating tuberculosis is one of the reasons for renewed extensive research on the discovery of a new generation of antibacterial drugs [1]. Considering the high antituberculosis activity of several steroids [2–4], it seemed interesting to seek new highly effective antituberculosis drugs among them.

The goal of the present work was to prepare the semicarbazone and isonicotinoylhydrazone of 5α-pregn-2-en-20-one and to study the antituberculosis activity of several steroids.

We synthesized earlier 5α-pregn-2-en-20-one and its derivatives from an intermediate in the conversion of tigogenin, 3β-hydroxy-5α-pregn-16-en-20-one acetate [5, 6]. Pregnenolone acetate (**1**) was converted into epiandrosterone acetate, base hydrolysis of which in MeOH gave epiandrosterone [7]. Jones oxidation in acetone of the 3β-hydroxy group [8] produced 5α-androstan-3,17-dione (**3**) in 87% yield. Androst-4-en-3,17-dione (**4**) was produced by microbiological cleavage of sitosterol [9].

Condensation of ketosteroids **2**–**4** with semicarbazide or isonicotinoylhydrazide in EtOH in the presence of CH_3COOH synthesized semicarbazones **5–7** and hydrazones **8–10**.



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The structures of the known and previously unreported compounds were confirmed by elemental analysis and IR and NMR spectra.

IR spectra of isonicotinoylhydrazones **8–10** contained characteristic absorption bands for NH stretching vibrations in the range 3380–3320 cm^{-1}; an NHC=O carbonyl, 1710–1650; a C=N bond, 1635–1625; and C–C and C–N bonds of a pyridine ring, 1535–1520. Semicarbazones **5–7** showed stretching vibrations of NH_2 in the range 3410–3390 cm^{-1}; of C=N bonds, 1640–1625; and of HN–C=O groups, 1740–1710.

NMR spectra of **5–10** exhibited resonances for the C-18 angular methyls as singlets in the range δ 0.60-0.94 ppm; for C-19 methyls, 0.78–1.17; for C-21 methyls of **5** and **8** as singlets, 1.83 and 1.74; and for vicinal C_2=C_3 protons as a complicated multiplet, 5.50–5.63. Resonances of pyridine protons of hydrazones **8**–**10** appeared at 7.50–8.71 as two doublets; of NH protons, at 8.91–10.96 as singlets. Resonances of NH_2 protons of semicarbazones **5**–**7** appeared as a broad singlet in the range 4.80–6.18 ppm.

Herein results of biological tests of ketosteroids **2**–**4**, semicarbazones **5–7**, and isonicotinoylhydrazones **8**–**10** and derivatives of modified epiandrosterone that were synthesized by us earlier, i.e., thiosemicarbazone **11** and nicotinoyl **12** and isonicotinoyl **13** hydrazones of 3β-(1-adamantoyl)-5α-androstan-17-one [10] are presented.

Antituberculosis activity was studied using the TAACF program (USA) for the discovery of new antituberculosis agents. Preliminary screening of **5**–**13** against *M. tuberculosis* strain H_{37}Rv in BACTEC 12B medium was carried out using the Microplate Alamar Blue Assay (MABA) [11]. The *in vitro* tests found low inhibiting activity for hydrazones **12** and **13** and thiosemicarbazone **11** at a concentration of 6.25 μg/mL. Compounds **6** and **7** were inactive. Steroids **8**–**10**, which exhibited high activity, were selected for subsequent study at lower concentrations relative to the same *M. tuberculosis* strain. Results of the antituberculosis tests of **5**-**13** are given below:*

Compound	*Inhibition*, %		*Compound*	*Inhibition*, %	
	stage I	stage II		stage I	stage II
5	16		**10**	96	96
6	0		**11**	35	
7	0		**12**	49	
8	96	96	**13**	41	
9	95	95			

*Minimum inhibiting concentration for **5**–**7** and **11**–**13** was >6.25 μg/mL; for **8**–**10**, <6.25 in stage I and <0.1 μg/mL in stage II.

The results provided good justification for continued research to seek new antituberculosis agents among steroids.

EXPERIMENTAL

IR spectra were taken in KBr pellets on a Thermo Nicolet Avatar-370 spectrometer; PMR spectra, in $CDCl_3$ and DMSO-d_6 on a Bruker AM-400 (400 MHz) spectrometer. Melting points were measured on a heated Boetius stage. The course of reactions and purity of synthesized compounds were monitored using TLC on Silufol UV-254 plates and benzene:acetone (6:1). Chromatograms were detected using phosphomolybdic acid solution (10%) in EtOH with subsequent heating. Elemental analysis was performed on an Elementar Vario CHNS instrument. Elemental analyses of all compounds agreed with those calculated. Pregnenolone acetate was prepared from tigogenin using the NH_4Cl–Py complex as a catalyst. This enabled the synthesis to be carried out in one step without autoclaving [5].

5α-Pregn-2-en-20-one Semicarbazone (5). A mixture of ketosteroid **2** (0.2 g, 0.66 mmol) and semicarbazide hydrochloride (0.1 g, 0.9 mmol) in EtOH (10 mL) was refluxed for 4 h (TLC monitoring). The precipitate that formed on cooling was filtered off and washed with H_2O. Yield of crude product 0.21 g (87%), mp 252–254°C (EtOH). IR spectrum (ν, cm^{-1}): 3410 (NH_2), 1740 (C=O), 1640 (C=N). PMR spectrum (δ, ppm): 0.60 (3H, s, 18-CH_3), 0.78 (3H, s, 19-CH_3), 1.83 (3H, s, 21-CH_3), 4.80 (1H, br.s) and 5.90 (1H, br.s, NH_2), 5.51–5.63 (2H, m, CH=CH), 7.54 (1H, s, NH).

Compounds **6** and **7** were prepared analogously.

5α-Androstan-3,17-dione disemicarbazone (6), mp 310–313°C (EtOH). IR spectrum (ν, cm^{-1}): 3420 (NH_2), 1710 (C=O), 1625 (N=C). PMR spectrum (δ, ppm): 0.85 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 6.03 (2H, br.s) and 6.07 (2H, br.s, NH_2), 8.97 (1H, s, NH on C-3), 8.65 (1H, s, NH on C-17).

Androst-4-en-3,17-dione disemicarbazone (7), mp 292–294°C (EtOH). IR spectrum (ν, cm^{-1}): 3400 (NH_2), 1710 (C=O), 1635 (N=C). PMR spectrum (δ, ppm): 0.83 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 5.74 (1H, s, H-4), 6.07 (2H, br.s) and 6.18 (2H, br.s, NH_2), 9.03 (1H, s, NH on C-3), 8.68 (1H, s, NH on C-17).

5α-Pregn-2-en-20-one Isonicotinoylhydrazone (8). A mixture of ketosteroid **2** (0.2 g, 0.66 mmol), isoniazide (0.1 g, 0.81 mmol), and HOAc (0.5 mL) in EtOH (10 mL) was refluxed for 5 h. The precipitate that formed on cooling was filtered off and washed with H_2O to afford the hydrazone (0.25 g, 92%), mp 154–156°C (EtOH). IR spectrum (ν, cm^{-1}): 3320 (NH), 1650 (NHC=O), 1625 (C=N), 1530 (Py ring). PMR spectrum (δ, ppm): 0.77 (3H, s, 18-CH_3), 0.79 (3H, s, 19-CH_3), 1.74 (3H, s, 21-CH_3), 5.50-5.63 (2H, m, CH=CH), 7.69 (2H, br.s) and 8.72 (2H, br.s, Py ring), 8.91 (1H, s, NH).

Compounds **9** and **10** were prepared analogously.

5α-Androstan-3,17-dione diisonicotinoylhydrazone (9), mp 276–278°C (MeOH) (lit. [12] mp 275–277°C). IR spectrum (ν, cm^{-1}): 3380 (NH), 1700 (C=O), 1635 (N=C), 1530 (Py ring). PMR spectrum (δ, ppm): 0.91 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 10.85 (1H, s, NH on C-3), 10.44 (1H, s, NH on C-17), 7.59 (2H, m) and 7.61 (2H, m, H-2,6 Py ring), 8.64 (2H, m) and 8.70 (2H, m, H-3,5 Py ring).

Androst-4-en-3,17-dione diisonicotinoylhydrazone (10), mp 292–294°C (MeOH) (lit. [12] mp 293–296°C). IR spectrum (ν, cm^{-1}): 3370 (NH), 1710 (C=O), 1630 (C=N), 1525 (Py ring). PMR spectrum (δ, ppm): 0.94 (3H, s, 18-CH_3), 1.1 (3H, s, 19-CH_3), 5.91 (1H, s, H-4), 10.96 (1H, s, NH on C-3), 10.72 (1H, s, NH on C-17), 7.54 (2H, m) and 7.74 (2H, m, H-2,6 Py ring), 8.64 (2H, m) and 8.71 (2H, m, H-3,5 Py ring).

Compounds **5**–**10** and adamantane-containing 5α-steroids **11**–**13** were tested using the TAACF program of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. Preliminary activity screening of the compounds at a concentration of 6.25 μg/mL against a *M. tuberculosis* strain (ATCC 27294) in BACTEC 12B medium was performed using the MABA.

Compounds exhibiting inhibiting activity <90% at this concentration were excluded from further testing. Compounds exhibiting anti-TB activity were studied in the second stage at lower concentrations against the same *M. tuberculosis* strain.

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