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SYNTHESIS AND ANTIVIRAL ACTIVITY OF SEVERAL *N*-CONTAINING 5α-STEROIDS

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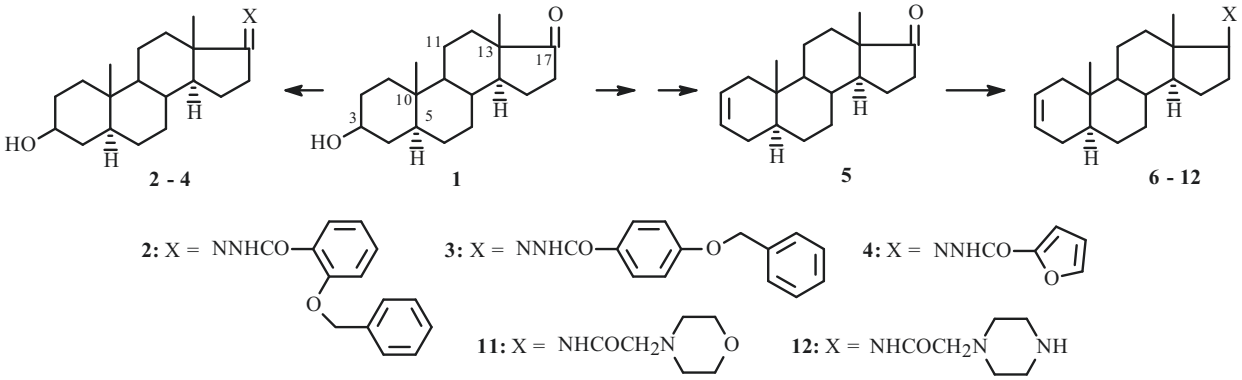
A study of the antiviral activity of several new hydrazones and amines and amides of 5α-steroids that were synthesized by us earlier found highly and moderately active compounds. The structures of the synthesized compounds were proven using IR, PMR, ^{13}C NMR, and mass spectral data.

Keywords: 5α-steroids, synthesis, hydrazides, hydrazones, amines, amides, antiviral activity.

Broad spectra of biological activity make steroids and their derivatives promising for discovering new efficacious drugs [1–3]. The high antibacterial, antiviral, and antitumor activity of hydrazono-, amino-, and amido-derivatives of the androstane, estrane, and cholestane series [4–8] and the expanded scope of their synthetic analogs helped to find new biological properties of these compounds.

In continuation of research on the synthesis of bioactive 5α-steroids from epiandrosterone **1** [9, 10], a transformation product of tigogenin, hydrazones **2–4** were obtained.



6: X = NH_2; **7:** X = $N(CH_3)_2$; **8:** X = $NHCOCH_3$; **9:** X = $NHCOCH_2Cl$; **10:** X = $NHCOCH_2N(C_2H_5)_2$

Herein we report results for the antiviral activity of synthesized **2–4** and **7–12** that were previously obtained from androst-2-en-17-one (**5**) and 17β-amino-5α-androst-2-ene (**6**) [11–13].

Starting 5α-androstanolone (**1**) was synthesized by the literature method [14]. Acid-catalyzed condensation of it with hydrazides of 2- and 4-benzyloxybenzoic and 2-furanoic acids produced hydrazones **2–4**. Epiandrosterone **1** was converted into ketone **5** and then into amine **6** or into 17β-(*N*,*N*-dimethylamino)-5α-androst-2-ene (**7**). Amides **8–12**, i.e., 17β-acetamido-5α-androst-2-ene (**8**), 17β-chloroacetylamino-5α-androst-2-ene (**9**), 17β-diethylaminoacetylamino-5α-androst-2-ene (**10**), 17β-morpholinoacetylamino-5α-androst-2-ene (**11**), and 17β-piperazinoacetylamino-5α-androst-2-ene (**12**), were synthesized from aminosteroid **6**.

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The structures of the synthesized compounds **2**, **3** were proven using IR, PMR, and mass spectra. IR spectra of hydrazones **2** and **3** contained characteristic absorption bands for NH and OH stretching vibrations at 3480–3310 and 3460–3305 cm^{-1} and for amide carbonyl stretching vibrations at 1649 and 1647 cm^{-1}. Characteristic frequencies of C=N bonds were observed at 1601 and 1608; aromatic C=C bonds, 1542 and 1501 cm^{-1}. The C–O ether bond gave bands at 1227 and 1250 cm^{-1}, respectively.

PMR spectra (DMSO-d_6) of **2** and **3** showed resonances for angular 18-CH_3 at δ 0.78 and 0.83 ppm; 19-CH_3, 0.80 and 0.90 ppm as singlets; 3α-H, at 3.33 ppm as multiplets; 3β OH protons, at 3.91 and 3.90 ppm as doublets with J = 4.5 and 4.4 Hz, respectively. Benzyloxy methylene protons were found at 5.20 and 5.14 ppm as singlets; aromatic protons, at 6.96–8.10 ppm. Weak-field NH protons appeared at 9.94 and 9.77 ppm, respectively.

The structure of **4** was confirmed by PMR, ^{13}C NMR (in $CDCl_3$), and mass spectra. The PMR spectrum of **4** had angular 18-CH_3 and 19-CH_3 at 0.84 and 0.94 ppm as singlets; 3α-H, 3.60 as a multiplet; furan-ring proton, 7.63–6.52 ppm (br); and NH, 8.70 ppm. ^{13}C NMR spectra contained peaks for furan-ring C atoms at 146.5–112.2 ppm; amide C=O and hydrazone C=N, 166.2 and 165.5 ppm, respectively. The molecular ion *m/z* $[M + H]^+$ 399.2 corresponded to $C_{24}H_{35}N_2O_3$.

Antiviral activity of **2–4** and **7–12** was studied at the National Institute of Allergy and Infectious Diseases (NIAID), University of Utah, USA. Studies on Vero 76 cell culture for poliovirus (strain Type 3, WM-3) showed that **3** had high antiviral activity; hydrazones **2** and **4**, moderate activity. The other steroids **7–12** were inactive. Steroids **7** and **8** exhibited moderate activity against Venezuelan equine encephalitis virus (strain TC-83); amide **12**, weak activity against Sars corona virus (strain Urbani). All other compounds were inactive. Only hydrazone **3** showed weak activity against Rift Valley fever virus (Vero 76 cell culture, strain MP-12); only **4**, against Respiratory syncytial virus (MA-104 cell culture, strain A-2). Compounds **2–4** and **7–12** were inactive against Influenza A virus H_1N_1, Takaribe virus, and Dengue virus (MDCK, Vero, Vero 76 cell cultures; strains California 07.2009, TRVL-11573, and Type-2, NewGuinea C, respectively).

EXPERIMENTAL

PMR were recorded in DMSO-d_6 and $CDCl_3$ with $SiMe_4$ internal standard on a Bruker Avance 400 spectrometer (400.13 MHz). IR spectra were taken from KBr pellets on a Varian 660 FT-IR spectrometer. Mass spectra were obtained on an Agilent 1100 series HPLC-APCIMS (positive mode) using an Inertsilprep-ODS column (6.0 × 250 mm) with elution by H_2O–MeCN (20:80). Melting points were determined on a Nagema apparatus. The course of reactions and purity of synthesized compounds were monitored by TLC on Silufol UV254 plates (Kavalier, Czechoslovakia) using C_6H_6–Me_2CO (4:1, 10:1). Chromatograms were detected by phosphomolybdic acid solution (10%) in EtOH followed by heating.

General Method for Synthesizing 17-Hydrazono Derivatives of 5α-Androstan-3-ols 2–4. A mixture of **1** (0.1 g, 0.35 mmol) and the appropriate hydrazide (0.55 mmol) in EtOH (10 mL) with a catalytic amount of AcOH was refluxed for 6–10 h and cooled to room temperature. The resulting precipitate was filtered off, rinsed with H_2O and Et_2O, dried, and crystallized from EtOH.

5α-Androstan-3β-ol-17-one 2-Benzyloxybenzoylhydrazone (2). Yield 70%, mp 232–233°C. IR spectrum (KBr, ν, cm^{-1}): 3480–3310 (NH, OH), 1649 (NHC=O), 1601 (C=N), 1542 (C=CAr), 1227 (C–O eth.). ^1H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 0.78 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 3.33 (1H, m, H-3), 3.91 (1H, d, J = 4.5, OH), 5.20 (2H, s, OCH_2), 7.09 (1H, t, J = 7.5, H-Ar), 7.23 (1H, d, J = 8.3, H-Ar), 7.34–7.56 (6H, m, H-Ar), 8.10 (1H, dd, J = 7.8, 1.8, H-Ar), 9.94 (1H, s, NH). LC-MS, *m/z* $[M + H]^+$ 515.3. $C_{33}H_{42}N_2O_3$, MM 514.3.

5α-Androstan-3β-ol-17-one 4-Benzyloxybenzoylhydrazone (3). Yield 73%, mp 210–212°C. IR spectrum (KBr, ν, cm^{-1}): 3460–3305 (NH, OH), 1647 (NHC=O), 1608 (C=N), 1501 (C=CAr), 1250 (C–O eth.). ^1H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 0.83 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 3.33 (1H, m, H-3), 3.90 (1H, d, J = 4.4, OH), 5.14 (2H, s, OCH_2), 6.96 (2H, m, C_6H_4), 7.25–7.44 (5H, m, C_6H_5), 7.80 (2H, m, C_6H_4), 9.77 (1H, s, NH). LC-MS, *m/z* $[M + H]^+$ 515.3. $C_{33}H_{42}N_2O_3$, MM 514.3.

5α-Androstan-3β-ol-17-one 2-Furanylhydrazone (4). Yield 62%, mp 240–242°C. ^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.84 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 3.60 (1H, m, H-3), 6.52 (1H, br.s, H-4′ fur.), 7.48 (1H, br.s, H-3′ fur.), 7.63 (1H, br.s, H-5′ fur.), 8.70 (1H, br.s, NH). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.2, 16.8, 20.6, 23.2, 25.9, 28.4, 31.4, 31.6, 33.8, 34.8, 35.0, 35.5, 36.8, 38.0, 44.7, 53.3, 54.4, 71.1 (C-3), 112.2 (C-4′ fur.), 116.8 (C-3′ fur.), 143.7 (C-5′ fur.), 146.5 (C-2′ fur.), 165.8 (C=N), 166.2 (C=O). LC-MS, *m/z* $[M + H]^+$ 399.2. $C_{24}H_{34}N_2O_3$, MM 398.2.

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