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SYNTHESIS AND ANTITUBERCULOSIS ACTIVITY OF CERTAIN STEROIDAL DERIVATIVES OF THE 5α-SERIES

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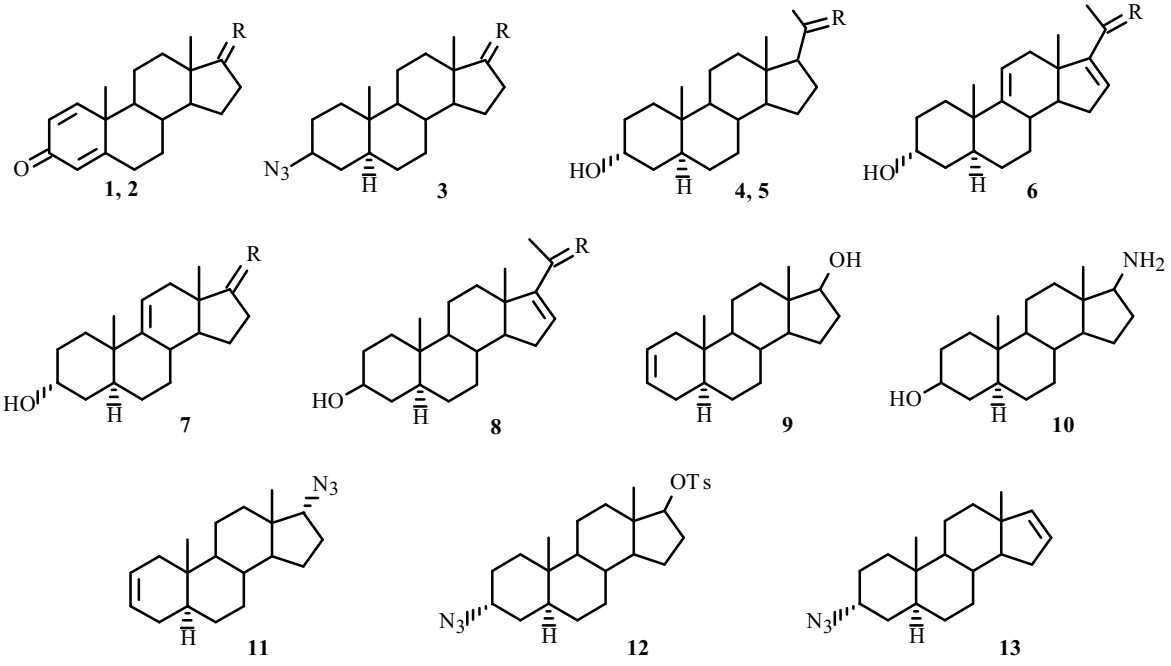
The antituberculosis activity of several steroidal compounds of various structure was studied. Several compounds that were active toward M. tuberculosis *were found in* in vitro *tests.*

Key words: antituberculosis activity, isonicotinoylhydrazones, thiosemicarbazones, steroids.

We previously reported the synthesis and biological activity of several new isonicotinoylhydrazones of 5α-steroids that exhibited high antituberculosis activity in *in vitro* tests [1, 2]. In continuation of the search for new highly effective antituberculosis compounds in this same series, we recently synthesized new hydrazones of saturated and unsaturated ketosteroids [3]. Considering that certain steroids themselves exhibit high activity toward *M. tuberculosis* [4-6], we studied hydrazones of steroids (**1-8**) and certain steroid derivatives **9-13** that we prepared earlier [7-9]. In addition, 17α-azido-3β-(4-methylphenylsulfonyloxy)-5α-androstane (**14**) was synthesized starting from epiandrosterone acetate (**15**) via its transformation into 17α-azido-3β-acetoxy-5α-androstane (**16**) and then into 17α-azido-5α-androstan-3β-ol (**17**) and its tosylated derivative (**14**). The structures of the synthesized compounds were confirmed by IR and NMR spectroscopy and mass spectrometry.



2, 5, 6 - 8: R = $NNHCSNH_2$; **1, 3, 4:** R = $NNHCOC_6H_5N$

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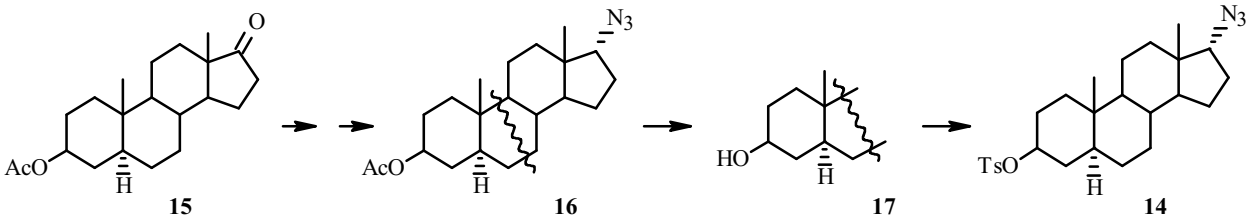

Herein we report results from biological tests of the following synthesized steroid derivatives: hydrazones of androst-1,4-dien-3,17-dione (**1**, **2**), 3β-azido-5α-androstan-17-one (**3**), 3α-hydroxy-5α-pregnan-20-one (**4**, **5**), 3α-hydroxy-5α-pregn-9(11),16-dien-20-one (**6**), 3α-hydroxy-5α-androst-9(11)-en-17-one (**7**), and 3β-hydroxy-5α-pregn-16-en-20-one (**8**) [4] in addition to a ketosteroid (**9**), an amine (**10**) and azides (**11**-**14**).

The antituberculosis activity of **1**-**14** was studied in a series of dilutions in Middlebrook 7H9 medium using *M. tuberculosis* H37Rv as the test culture. Isonicotinoylhydrazones **3** and **4**, thiosemicarbazone **6**, azides **11** and **12**, and aminosteroid **10** exhibited high activity toward *M. tuberculosis* H37Rv in *in vitro* tests at all studied concentrations (5, 10, 25, 50, and 100 μg/mL). Compounds **5** and **9**, **7**, and **8** and **13** were active at concentrations of 10, 25, and 50 μg/mL, respectively. Compound **14** exhibited activity only at a concentration of 100 μg/mL whereas hydrazones **1** and **2** were inactive against *M. tuberculosis* H37Rv even at 100 μg/mL. The antituberculosis activity of steroidal azides has not been reported. This active class of compounds is reported for the first time. The biological tests provide a good impetus for more extensive study of the aforementioned steroidal derivatives and continuation of the search for new highly effective compounds in this series.

EXPERIMENTAL

Melting points were determined on a Gallenkamp block. IR spectra in KBr disks were recorded on a Magna-IR Spectrometer 550. NMR spectra were obtained on a Bruker AC 500 instrument (operating frequency 500 MHz for ^{1}H). Chemical shifts of protons are given on the δ scale with TMS internal standard and $CDCl_3$ solvent. Mass spectra were recorded in a MAT-112 GC—MS (ionizing electron energy 70 eV, ionization chamber temperature 180°C, direct sample introduction into the source). Elemental analyses was performed on a Perkin—Elmer CHN 2004 instrument and agreed with those calculated. The course of reactions and purity of products were monitored by TLC on plates (60GF-254, Merck) using benzene:acetone (15:1). Spots were developed by spraying with phosphomolybdic acid solution (10%) in ethanol with subsequent heating.

17α-Azido-5α-androstan-3β-ol (17). A mixture of 17α-azido-3β-acetoxy-5α-androstane (**16**, 5 g, 13.9 mmol) and KOH (0.7 g, 17.5 mmol) in methanol (40 mL) was refluxed for 10 min, cooled, and poured into water (100 mL). The product (4.3 g) was separated and crystallized from benzene:hexane (1:4) to afford **17** (4.2 g, 96%), mp 136-138°C. IR spectrum (KBr, ν, cm^{-1}): 3300-3500 (OH), 2105 (N_3). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.78 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 3.53 (1H, d, J = 6.5, H-17β), 3.59 (1H, m, H-3α). Mass spectrum (EI, 70 eV, *m*/*z*, *I*, %): 289 (100) $[M - N_2]^+$.

17α-Azido-3β-(4-methylsulfonyloxy)-5α-androstane (14). A solution of **17** (3 g, 9.44 mmol) in freshly distilled pyridine (30 mL) at 0°C was treated with *p*-toluenesulfonylchloride (4.45 g, 23.35 mmol), held at 20°C for 24 h, poured into icewater (200 mL), and filtered. The product (4.13 g) was separated and crystallized from benzene:hexane (1:2) to afford **14** (4.09 g, 92%), mp 153-155°C. IR spectrum (KBr, ν, cm^{-1}): 2105 (N_3). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, CH_3-18), 0.77 (3H, s, CH_3-19), 2.44 (3H, s, CH_3-Ar), 3.47 (1H, d, J = 6.5, H-17β), 4.41 (1H, m, H-3α), 7.32 (1H, d, J = 8.5, H-Ar), 7.78 (1H, d, J = 8.5, H-Ar). Mass spectrum (EI, 70 eV, *m*/*z*, *I*, %): 471 (100) $[M]^+$.

The antituberculosis activity was determined toward *M. tuberculosis* H37Rv. Middlebrook 7H9 medium was prepared in bottles and sterilized by autoclaving. Growth additive ADC (argininedecarboxylase) containing fraction V of bovine albumin, dextrose, and catalase was added to each bottle under sterile conditions. A sterile suspension of each tested compound (10 mg/mL) was prepared in the appropriate solvent. Each compound was tested at concentrations of 5-100 μg/mL that was added to the bottles with Middlebrook 7H9 medium and a suspension of *M. tuberculosis* culture (10^5 cells/mL). The medium was stored for at least four weeks at 37°C in a moist chamber. The reference preparations were ciprofloxacin (5 μg/mL), streptomycin (7.5 μg/mL), or pyrazinamide (7.5 μg/mL). Medium and growth additive were used as separate controls.

Growth or possible contamination was checked periodically in each bottle. A smear that was colored by dye Z-N (Ziehl—Neelsen) was taken from each bottle with cloudiness at the end of the fourth week in order to confirm the presence of *M. tuberculosis*. Media in which *M. tuberculosis* cells were present were classified as resistant to the studied compounds. Media in which no bacteria were found were considered sensitive.

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