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SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF SOME STEROIDAL DERIVATIVES OF TIGOGENIN

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Some 5α-type steroidal derivatives of the steroidal sapogenin tigogenin were synthesized. The structures of the synthesized derivatives were characterized by spectral methods and elemental analysis. The antituberculosis activity of the compounds toward Mycobacterium tuberculosis *strain H37Rv was studied in vitro (Microplate Alamar Blue Assay) in BACTEC 12B medium and was compared with that of isoniazide. Some of the synthesized compounds exhibited high (92-98%) antimycobacterial activity.*

Key words: antimycobacterial activity, 5α-steroidal hydrazones, 5α-aminosteroids.

Changes of various social, medical, and economic factors at the end of the 1980s caused tuberculosis to start again to spread. The emergence of resistant strains of the disease that exposed the imperfection of the modern arsenal of drugs enhanced the rebound of tuberculosis [1]. The need arose to create new drugs that could be used in shorter treatment courses than existing drugs in order to treat resistant and latent forms of the disease.

It was recently proposed that the mechanism of resistance to isoniazide, which remains to this day the first-line drug, is related to mutation or deletion of peroxidase catalase KatG and that antibacterial resistance to the drug can be overcome by synthesizing new isoniazide derivatives, in particular, by preparing biologically different forms of this drug, namely hydrazones. Nevertheless, isonicotinoylhydrazones (INH) themselves are viewed as compounds with potential antituberculosis activity [2].

We reported previously on the antimycobacterial activity of some 5α-steroidal INH *in vitro* [3-5]. It was found that some of them were just as active toward *M. tuberculosis* as isoniazide. Taking this into account, we synthesized various new steroidal INH [6] and other derivatives. The goal of the present study was to compare the antimycobacterial activity of the previously and newly synthesized compounds.

Starting ketosteroids (**2**-**9**) were prepared from tigogenin (**1**) that was isolated from *Yucca gloriosa* L. by the published method [3-8]. Steroidal hydrazones (**10**-**18**) were prepared by condensation of isoniazide [3, 5, 6], *m*-nitrobenzoic acid hydrazide, and *m*-bromobenzoic acid hydrazide with steroidal skeletons (**2**-**9**). An aminosteroid (**19**) was synthesized from epiandrosterone acetate (**6**) as before [7] (Scheme 1).

The structures of the newly synthesized compounds **17** and **18** were confirmed by IR, MS, PMR, and ^{13}C NMR spectral data.

IR spectra of **17** and **18** exhibited absorption bands in the range 3400-3360 cm^{-1} that were characteristic for NH. Bands at 2946-2924 were assigned to the aliphatic part. The absorption band of HNC=O appeared at 1736 and 1705, respectively. The C=N band in both instances appeared at 1641; the aromatic part, 1550. The IR spectrum of **17** showed absorption bands at 1518 and 1335 (Ar–NO_2); of **18**, 750 (Ar–Br). Resonances of angular 18- and 19-methyls in PMR spectra of **17** and **18** were singlets at δ 0.82-0.88 ppm. The methyl of the C-3 acetyl resonated at 1.97; axial 3α-H of 3α-steroidal esters, as a multiplet at 4.60. Aromatic protons appeared as two doublets, one triplet, and a singlet. The NH group gave a broad singlet at 10.32-10.57 ppm.

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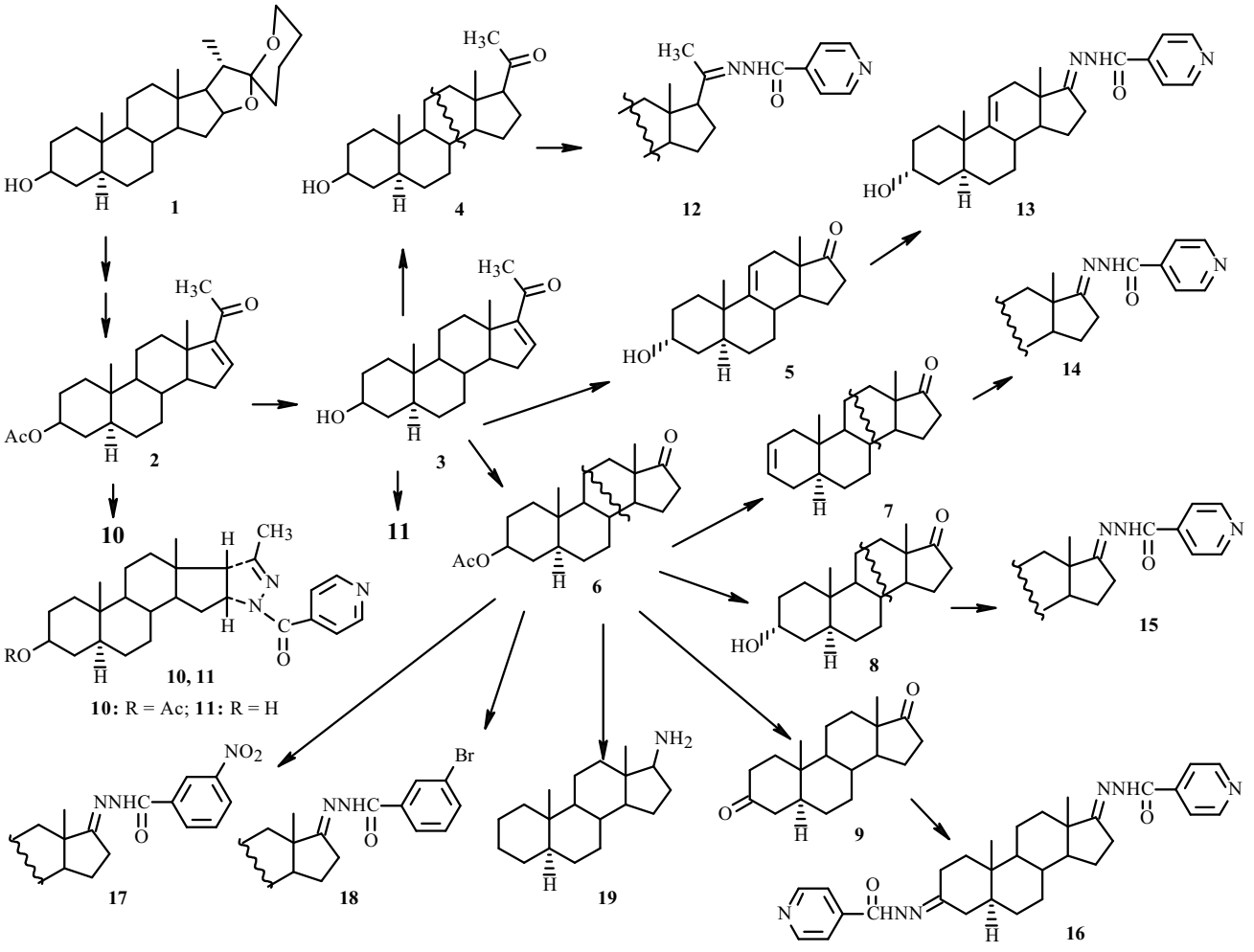
TABLE 1. Antimycobacterial Activity of Synthesized Compounds **10**–**19** *in vitro*

Compound	Inh[a], %	MIC, μg/mL	IC_{50}, μg/mL	SI[b]
10	98	>6.25	6.14	
11	92	0.78	>10.0	>12.82
12	94	0.1	1.97	19.7
13	94	0.39	9.65	24.74
14	98	0.39	>6.25	>160.3
15	94	0.39	>10.0	>25.64
16	92	0.78	>10.0	>12.8
17	20	–	–	–
18	28	–	–	–
19[c]	98	1.56	–	–
INH	–	0.025–0.05	>100	

[a]Against *M. tuberculosis* H37Rv at a concentration of 6.25 μg/mL; [b]SI = IC_{50}/MIC for *M. tuberculosis*; [c]SI not determined due to solubility problems.



Steroidal derivatives **10**-**19** were tested for antituberculosis activity. Aminosteroid **19** and INH **10**-**16** showed high (92-98%) inhibiting activity during preliminary screening. Hydrazones **17** and **18** at this stage were ineffective (%Inh = 20) and were not further investigated.

Results of the second stage of TAACF gave the lowest MIC (minimum inhibiting concentration) for INH: **12** (0.1 μg/mL), **14** (0.39), **15** (0.39), **13** (0.39), **16** (0.78), and **11** (0.78). However, these compounds exhibited low cytotoxicity toward VERO cells, showing a good selectivity index SI = IC_{50}/MIC > 12 (Table 1). Despite the high inhibiting activity of

aminosteroid **19**, its SI could not be found due to solubility problems (even at a concentration of 1 μg/mL the compound was insoluble in DMSO).

The significant antituberculosis activity shown by some of the synthesized INH and aminosteroids makes them attractive for their further modification and suggests that these compounds are promising for creating new antituberculosis agents.

EXPERIMENTAL

Melting points were determined on a Gallenkamp block. IR spectra in KBr disks were recorded on a Magna-IR Spectrometer 550 instrument. PMR spectra were obtained on a Bruker AC 500 instrument (operating frequency 500 MHz for ^{1}H and 125 MHz for ^{13}C). Chemical shifts of protons in DMSO-d_6 are given on the δ scale in ppm with TMS as an internal standard. Mass spectra were recorded in a Finnigan AQA Navigator instrument (EI, 70 eV). Elemental analysis (C, H, N) was performed on a Perkin—Elmer CHN 2004 instrument. Analyses of all compounds agreed with those calculated to within ±0.4%. The course of reactions and purity of products were monitored by TLC on Merck silica gel plates (0.25 mm, 60GF-254). Spots were detected in UV light and were treated by spraying with phosphomolybdic acid in ethanol (10%) with subsequent heating.

Epiandrosterone Acetate *m*-Nitrobenzoylhydrazone (17). A mixture of epiandrosterone acetate (1 g, 3.0 mmol), *m*-nitrobenzoic acid hydrazide (1.08 g, 6.0 mmol), and acetic acid (1 mL) in ethanol (10 mL) was refluxed for 12 h and cooled to room temperature. The resulting precipitate was filtered off, washed with water, and dried. Recrystallization from ethanol afforded **17** (1.34 g, 90%), mp 215-218°C (ethanol). IR spectrum (ν, cm^{-1}): 3360 (NH), 1736 (NH–CO), 1641 (C=N, hydrazone), 1620 (ester C=O), 1550 (aromatic ring), 1518 and 1335 (Ar–NO_2).

PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.83 (3H, s, CH_3-10), 0.88 (3H, s, CH_3-13), 1.97 (3H, s, $COCH_3$), 4.6 (1H, m, 3-Hα), 7.8 (1H, t, H-5′), 8.23 (1H, d, J = 7.1, H-6′), 8.4 (1H, d, J = 7.3, H-4′), 8.59 (1H, s, H-2′), 10.57 (1H, br.s, NH).

^{13}C NMR spectrum (δ, ppm): 12.37 (CH_3), 17.27 (CH_3), 19.08 (CH_3), 73.29 (A–O–C), 122.90-136.15 (5C of aromatic ring), 148.04 (C–NO_2), 161.76 (NHC=O), 166.51 (C=N), 170.25 (CH_3C=O). Mass spectrum (*m*/*z*, %): 495 (15) $[M]^+$.

Epiandrosterone acetate *m*-bromobenzoylhydrazone (18) was prepared analogously to **17** from epiandrosterone acetate and *m*-bromobenzoic acid hydrazide. Yield 93%, mp 218-220°C (ethanol). IR spectrum (KBr, ν, cm^{-1}): 3420 (NH), 1705 (NH–CO), 1641 (C=N, hydrazone), 1620 (ester C=O), 1550 (aromatic ring), 1750 (Ar–Br).

PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.82 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 1.97 (s, 3H, $COCH_3$), 4.6 (1H, m, 3-Hα), 7.44 (1H, t, H-5′), 7.75 (1H, d, J = 7.1, H-6′), 7.79 (1H, d, J = 7.5, H-4′), 7.95 (1H, s, H-2′), 10.32 (1H, br.s, NH).

^{13}C NMR spectrum (δ, ppm): 11.87 (CH_3), 16.78 (CH_3), 18.51 (CH_3), 72.80 (Ac–O–C), 121.45 (C–Br), 126.74-136.43 (5C of aromatic ring), 161.21 (NHC=O), 162.51 (C=N), 171.25 (CH_3C=O). Mass spectrum (*m*/*z*, %): 529 (24) $[M]^+$.

Antimycobacterial activity of **10**-**19** was evaluated *in vitro* according to the TAACF program coordinated by Southern Research Institute (Birmingham, AL, USA) under the direction of the National Institute of Allergy and Infectious Diseases. Preliminary screening of the activity of the compounds at a concentration of 6.25 μg/mL toward *M. tuberculosis* strain H37Rv (ATTCC 27294) in BACTEC 12B medium was performed using the Microplate Almar Blue Assay (MABA) test [9]. Compounds exhibiting inhibiting activity (%Inh) less than 90% at this concentration were not further investigated. In the second stage, MIC, cytotoxicity toward VERO cells, and selectivity index were determined. Some of the synthesized compounds exhibited high antituberculosis activity (Table 1).

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