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SYNTHESIS OF NOVEL STEROIDAL ISONICOTINYLHYDRAZONES AND THIOSEMICARBAZONES FROM TIGOGENIN

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A series of new isonicotinylhydrazones and thiosemicarbazones of various ketosteroids was synthesized. The structures of the compounds were confirmed by NMR and IR spectroscopy and mass spectrometry.

Key words: ketosteroids, isonicotinylhydrazones, thiosemicarbazones, synthesis.

The significant increase in the incidence and morbidity from tuberculosis since the start of the 1990s prompted the World Health Organization to regard this disease as a worldwide danger [1]. One of the factors leading to the increased incidence is the development of resistance in *Mycobacterium tuberculosis* toward existing medicinal preparations. Therefore, the search for new effective antituberculosis compounds has become urgent.

Isonicotinylhydrazones and thiosemicarbazones of various structure are known to exhibit antituberculosis activity [2-6]. Several derivatives of isoniazide and thiosemicarbazide based on tigogenin that were synthesized by us were highly active toward *M. tuberculosis* [7, 8].

In continuation of the search for new highly effective steroidal antituberculosis compounds, we synthesized a series of hydrazones of saturated and unsaturated ketosteroids, in particular, hydrazones of 3α-hydroxy-5α-pregnan-20-one, 3α-hydroxy-5α-pregn-9(11),16-dien-20-one, 3α-hydroxy-5α-androst-9(11)-en-17-one, 17β-hydroxy-5α-androstan-3-one, 3β-azido-5α-androstan-17-one, 3β-hydroxyandrost-5-en-17-one, and androst-1,4-dien-3,17-dione.

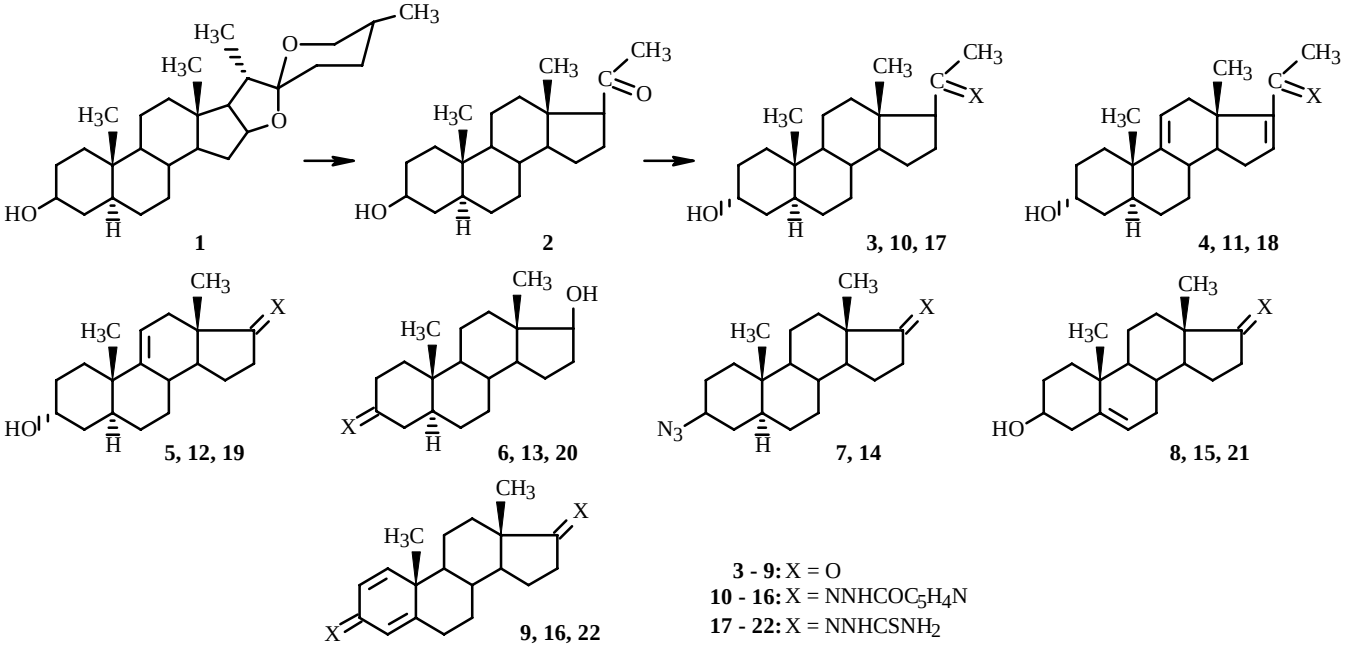

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TABLE 1. Physicochemical Properties of **10**-**22**

Compound	mp, °C	Yield, %	Emp. formula	$[M]^+$
10	285-287	90	$C_{27}H_{39}N_3O_2$	437
11	225 (dec.)	93	$C_{27}H_{35}N_3O_2$	433
12	295-297	97	$C_{25}H_{33}N_3O_2$	407
13	188-190	90	$C_{25}H_{35}N_3O_2$	409
	Lit.: 189-192 [16]			
14	205-207	90	$C_{25}H_{34}N_6O$	434
15	230-231	90	$C_{25}H_{33}N_3O_2$	407
	Lit.: 231-232 [3]			
16	310-312	90	$C_{31}H_{34}N_6O_2$	522
17	165-167	90	$C_{22}H_{37}N_3OS$	391
18	265 (dec.)	80	$C_{22}H_{33}N_3OS$	387
19	300 (dec.)	85	$C_{20}H_{31}N_3OS$	361
20	218-220	90	$C_{20}H_{33}N_3OS$	363
	Lit.: 217-220 [17]			
21	237-239	90	$C_{20}H_{31}N_3OS$	361
	Lit.: 237-238 [18]			
22	185-187	90	$C_{21}H_{30}N_6S_2$	430

The starting compound for synthesizing the 5α-ketosteroids was tigogenin (**1**), which was isolated from leaves of *Yucca gloriosa* cultivated in Georgia. Tigogenin was converted to ketosteroids (**2**-**7**) by previously described methods [9-15]. The configuration of the steroidal 3β-alcohol (**2**) and its 3α-epimer (**3**) was inverted as before [11], which involves formation of an intermediate *m*-iodobenzoate of this steroid in the presence of diethylazodicarboxylate, triphenylphosphine, and *m*-iodobenzoic acid in THF and its subsequent alkaline hydrolysis. The 9(11)-double bond was introduced into the steroidal system of **4** and **5** using radical selective chlorination at the C-9 position [12] with subsequent simultaneous dehydrochlorination and hydrolysis of the 9α-chlorides to give the 9(11)-unsaturated alcohols [13]. Dihydrotestoserone (**6**) was synthesized from epiandrosterone with formation of intermediate 3,3′-dimethoxy-5α-androstan-17-one [14]; 3β-azido-5α-androstan-17-one (**7**), through formation of epiandrosterone *p*-toluenesulfonate [15]. Unsaturated ketones **8** and **9** were purchased (Sigma).

Isonicotinylhydrazones **10**-**16** were prepared by boiling the appropriate ketones **3**-**9** with isonicotinic acid hydrazide in the presence of acetic acid; thiosemicarbazones **17**-**22**, from ketones **3**-**6** and **8** and **9** with thiosemicarbazide in ethanol.

The structures of **10**-**22** were confirmed by NMR and IR spectroscopy and mass spectrometry. The IR spectra of the isonicotinylhydrazones contained characteristic absorption bands for NH stretches at 3400-3100 cm^{-1}; HNC=O carbonyl, 1690-1640; C=N, 1660-1620; and pyridine C–C and C–N, 1550-1500. Absorption bands for NH stretches of thiosemicarbazones appeared at 3400-3100 cm^{-1}; C=N, 1660-1645; and thiocarbonyl, 1400-1300. Absorption bands of steroidal hydroxyl were found at 3400-3500 cm^{-1}.

PMR spectra of **10**-**12**, **14**, **16**-**19**, and **22** exhibited singlets for angular methyls on C-13 and C-10 at δ 0.61-1.17 ppm; pyridine protons, two doublets at δ 7.63-8.73. The NH signals were found as a singlet in the range δ 10.18-10.68 ppm. The axial 3β-protons of 3α-steroidal pregnane alcohols were typically broad singlets at δ 3.35-3.45 ppm. The ^{13}C NMR spectra of isonicotinylhydrazones showed two strong resonances for pyridine C atoms at δ 149.6-150.1 ppm and δ 121.4-122.8 ppm; the quaternary pyridine C atoms, δ 140.5; two weaker resonances for the hydrazone carbonyl at δ 176.2-183.2 ppm; and C=N, δ 163.1-168.9. The ^{13}C NMR spectra of thiosemicarbazones showed strong characteristic resonances for thiocarbonyl and hydrazone C atoms at δ 167.2-178.6 ppm and δ 154.8-166.7 ppm, respectively.

Mass spectra of the synthesized compounds exhibited peaks for molecular ions corresponding to the molecular masses of these compounds. The fragmentation patterns of **10**-**12** and **14** were identical. Decomposition produced ions with mass numbers for $[M - CO - C_5H_4N]^+$ at 331, 327, 301, and 328, respectively. The molecular ions of **17**-**19** fragmented to give ions with mass numbers 316, 312, and 286 for $[M - NHCSNH_2]^+$ and 302, 298, and 272 for $[M - NNHCSNH_2]^+$.

Tables 1 and 2 give the physicochemical properties of **10**-**22**. The antituberculosis activity of the synthesized compounds (**10**-**22**) is under investigation.

TABLE 2. PMR and IR Data for **10**-**12**, **14**, **16**-**19**, and **22**

Compound	PMR, δ, ppm	IR spectrum, ν, cm^{-1}
10	0.61 (3H, s, CH_3-10), 0.72 (3H, s, CH_3-13), 1.92 (3H, s, CH_3-20), 3.35 (br.s, C–OH), 3.82 (br.s, 1H, H-3), 7.7 and 8.7 (4H, dd, arom., J_{ortho} = 8.2 Hz, J_{meta} = 8.2 Hz), 10.68 (br.s, 1H, NH)	3418 (OH), 1663 (NHC=O), 1625 (C=N), 1527 (pyridine ring), 1296 (C-N)
11	0.86 (3H, s, CH_3-10), 0.87 (3H, s, CH_3-13), 1.23 (3H, s, CH_3-20), 3.45 (br.s, C-OH), 3.82 (1H, s, 3-H), 5.31 (1H, m, H-11), 7.76 and 8.73 (4H, dd, arom.., J_{ortho} = 8.2 Hz, J_{meta} = 8.2 Hz), 10.73 (1H, br.s, NH)	3440 (OH), 1680 (NHC=O), 1620 (C=N), 1590 (C=C), 1505 (pyridine ring), 1288 (C-N))
12	0.82 (3H, s, CH_3-10), 0.88 (3H, s, CH_3-13), 3.45 (br.s, C-OH), 3.80 (1H, s, H-3), 5.35 (1H, m, 10-H), 7.70 and 8.7 (4H, dd, arom., J_{ortho} = 8.8 Hz, J_{meta} = 8.8 Hz), 10.44 (br.s, 1H, NH)	3455 (OH), 1668 (NHC=O), 1622 (C=N), 1506 (pyridine ring), 1277 (C-N)
14*	0.85 ((3H, s, CH_3-10), 1.07 (3H, s, CH_3-13), 7.65 and 8.67 (4H, dd, arom., J_{ortho} = 5.84 Hz, J_{meta} = 5.85 Hz), 8.40 (br.s, 1H, NH)	3197 (NH_2), 2070 (-N=N^+=N-), 1688 (NHC=O), 1620 (C=N), 1526 (pyridine ring), 1259 (C-N)
16	0.95 (3H, s, CH_3-10), 1.17 (3H, s, CH_3-13), 6.33 (1H, d, C-2H, J = 10.1 Hz), 6.39 (s, 1H, H-4), 6.41 (1H, d, H-1, J = 10.14 Hz), 7.63 and 7.71 (4H, dd, arom. on C17, J_{ortho} = 5.52 Hz, J_{meta} = 5.52 Hz), 8.62 and 8.64 (4H, dd, arom. on C3, J_{ortho} = 5.54 Hz, J_{meta} = 5.54 Hz), 10.29 (1H, s, NH C-17), 10.99 (1H, s, NH C-3)	3339 (NH_2), 1640 (NHC=O), 1630 (C=N),1526 (pyridine ring), 1277 (C-N)
17	0.62 (3H, s, CH_3-10), 0.78 (3H, s, CH_3-13), 1.95 (3H, s, CH_3-20), 3.8 (1H, m, 3-H), 7.36 and 8.06 (NH_2), 9.86 (1H, s, NHC=S)	3427 (OH), 1645 (C=N), 1336 (C=S), 1296 (NHCS)
18	0.76 (3H, s, CH_3-10), 0.83 (3H, s, CH_3-13), 0.83 (3H, s, 21-CH_3), 3.45 (br.s, C-OH), 3.83 (1H, s, 3-H), 5.31 (1H, m, 11-H), 6.97 and 8.31 (NH_2), 10.06 (1H, s, NHC=S)	3430 (OH), 1649 (C=N), 1350 (C=S), 1300 (NHCS)
19	0.84 (3H, s, CH_3-10), 0.93 (3H, s, CH_3-13), 3.38 (br.s, C-OH), 3.80 (1H, s, 3-H), 5.35 (1H, m, 10-H), 7.32 and 7.92 (NH_2), 9.75 (1H, s, NHC=S)	3430 (OH), 3350 (NH_2), 1649 (C=N), 1358 (C=S), 1300 (NHCS)
22	0.84 (3H, s, CH_3-10), 1.06 (3H, s, CH_3-13), 5.92 (1H), 6.14 (1H), 7.15 (1H), 7.37 and 7.55 (NH_2 TSC, C-3), 7.98 and 8.60 (NH_2 in TSC on C-17), 9.81 (1H, s, NHC=S in TSC on C-3), 10.52 (1H, s, NHC=S in TSC on C-17)	3379 (NH_2), 1660 (C=N), 1351 (C=S), 1287 (NHCS)

Spectra were recorded in DMSO-d_6.
*Spectrum recorded in $CDCl_3$.

EXPERIMENTAL

Melting points were determined on a Gallenkamp block. IR spectra were recorded on a Magna-IR 550 Spectrometer in KBr disks. Mass spectra were measured in a Finnigan AQA Navigator instrument (EI, 70 eV). NMR spectra were obtained on a Bruker AC 500 instrument. Elemental analyses were performed on a Perkin—Elmer CHN 2004 instrument and agreed with those calculated for all compounds. The course of reactions and purity of products were monitored by TLC on Silufol 254 plates (Kavalier, Czech Rep.) using benzene:acetone (3:1). Spots were developed by spraying with phosphomolybdic acid (10%) in ethanol with subsequent heating.

3α-Hydroxy-5α-pregnan-20-one Isonicotinylhydrazone (10). A mixture of 3α-hydroxy-5α-pregnan-20-one (**3**, 1 g, 2.28 mmol), isoniazide (0.34 g, 2.5 mmol), and acetic acid (1 mL) in ethanol (20 mL) was boiled for 2 h and cooled to room temperature. The resulting precipitate was filtered off, washed with water, and crystallized from ethanol.

3α-Hydroxy-5α-pregn-9(11),16-dien-20-one (11), 3α-hydroxy-5α-androst-9(11)-en-17-one (12), 17β-hydroxy-5α-androstan-3-one (13), 3β-azido-5α-androstan-17-one (14), and 3β-hydroxyandrost-5-en-17-one (15) isonicotinylhdyrazones were prepared analogously to **10** from the corresponding ketones.

Androst-1,4-dien-3,17-dione Diisonicotinylhydrazone (16). A mixture of androst-1,4-dien-17-one (**9**, 1 g, 3.51 mmol), isoniazide (1.05 g, 7.73 mmol), and acetic acid (1 mL) in ethanol (20 mL) was boiled for 4 h and cooled to room temperature. The resulting precipitate was filtered off, washed with water, and crystallized from ethanol.

3α-Hydroxy-5α-pregnan-20-one Thiosemicarbazone (17). A mixture of 3α-hydroxy-5α-pregnan-20-one (**3**, 1 g, 2.28 mmol), thiosemicarbazide (0.20 g, 2.28 mmol), and acetic acid (1 mL) in ethanol (20 mL) was boiled for 1 h and cooled to room temperature. The resulting precipitate was filtered off, washed with water, and crystallized from ethanol.

3α-Hydroxy-5α-pregn-9(11),16-dien-20-one (18), 3α-hydroxy-5α-androst-9(11)-en-17-one (19), 17β-hydroxy-5α-androstan-3-one (20), and 3β-hydroxyandrost-5-en-17-one (21) thiosemicarbazones were prepared analogously to **17** from the corresponding ketones.

Androst-1,4-dien-3,17-dione Dithiosemicarbazone (22). A mixture of androst-1,4-dien-3,17-dione (**10**, 1 g, 3.51 mmol), thiosemicarbazide (0.40 g, 4.56 mmol), and acetic acid (1 mL) in ethanol (20 mL) was boiled for 2.5 h and cooled to room temperature. The resulting precipitate was filtered off, washed with water, and crystallized from ethanol.

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