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Pharmacochemistry

Synthesis of New 5α-Steroidal Hydrazones from Tigogenin

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(Presented by Academy Member Ether Kemertelidze)

In the present study potentially biologically active steroids, new hydrazones, semicarbazones, and 20-O-methyloxime have been synthesized from ketones of the 5α-pregnene, 5α-pregnane, and 5α-androstane series. The condensation reaction of ketones – 5α-pregn-16-en-3β-ol-20-one, 5α-pregnan-3β-ol-20-one and 5α-androstan-3β-ol-17-one – with various arylhydrazides, semicarbazide, and methoxyamine was carried out in ethanol with a catalytic amount of acetic acid. The starting ketones were made from tigogenin, an aglycone of steroidal saponins that is a domestic raw material for the synthesis of 5α-series steroids. Tigogenin was isolated from the Yucca gloriosa plant, which was introduced in Georgia. ^{1}H, ^{13}C NMR and mass spectra were used to confirm the structure of the newly obtained steroids. The cytotoxicity of these and previously synthesized hydrazones was investigated in vitro using the Rezazurin reduction test and Hoechst test aginst lung carcinoma (A-549), colorectal adenocarcinoma (DLD-1) and normal skin fibroblasts (WS-1) cell lines in comparison to etoposide. The results show that, of all the compounds studied, only p-methyl- and p-methoxybenzoylhydrazone 5α-pregnan-3β-ol-20-one are of particular interest since, unlike the others, they demonstrate activity comparable to etoposide. © *2022 Bull. Georg. Natl. Acad. Sci.*

pregnenolone, pregnanolone, epiandrosterone, hydrazide, hydrazone, oxime, 5α-steroids, cytotoxic activity

Scientists have been inspired to steroidal semicarbazones and hydrazones due to their antiviral, anticancer, and antiproliferative properties [1,2].

In recent years, there has been a surge of interest in introducing an oxime group into the steroidal nucleus in order to boost their biological effects [3]. Oxime esters are known to have variable cytotoxicity against different cancer cell lines. Compounds with the O-methyloxime and O-benzyloxime structures have better antiproliferative activity [4,5].

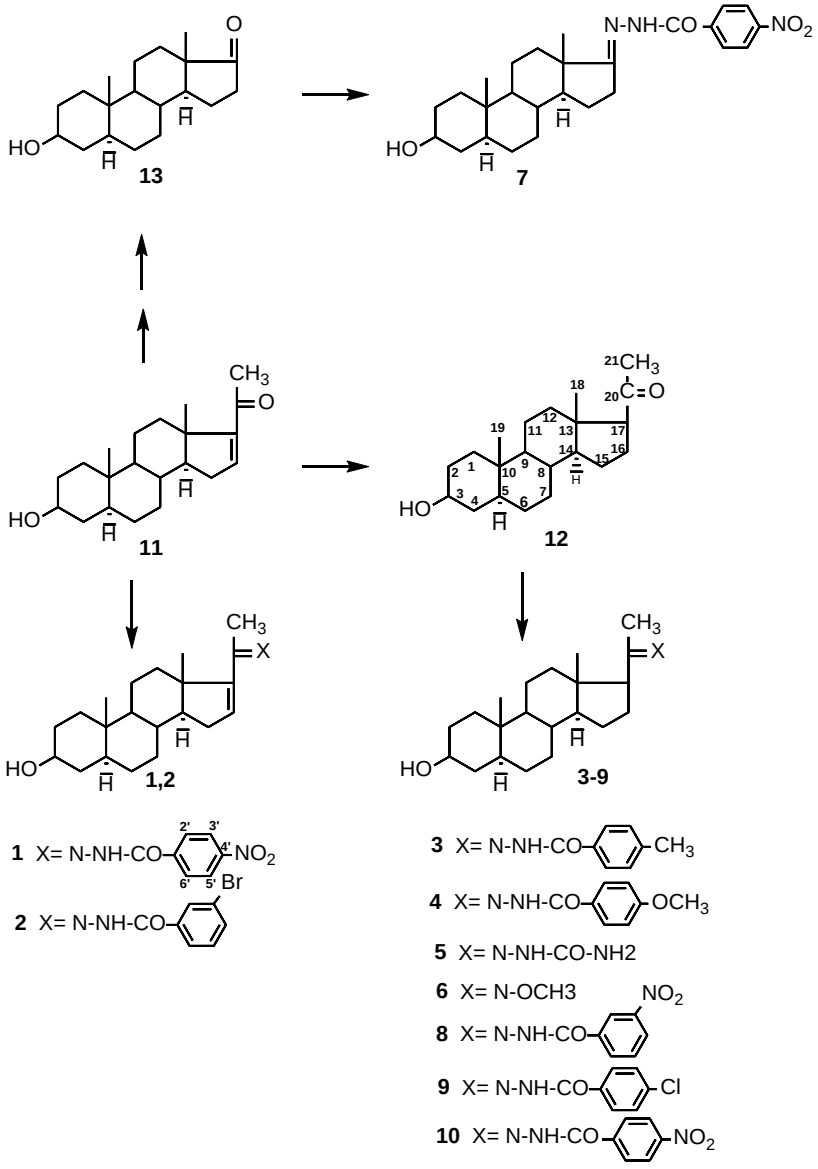
Some hydrazones and oximes of 5α-steroids that we previously synthesized on the basis of tigogenin demonstrated high antiviral, antimicrobial, and cytotoxic activity [6–10].

New nitrogen-containing compounds **1-7** were synthesized in the present study potentially bio-

logically active 5α-steroids. The following data on the cytotoxic activity of these and previously obtained by us [11] hydrazones: *m*-nitro-, *p*-chloro-, *p*-nitrobenzoylhydrazone of 5α-Pregnan-3β-ol-20-one **8-10** are presented in the paper.

Hydrazones **1, 2** were synthesized from 5α-pregnenolone **11**, steroids **3-6, 8-10** – from 5α-pregnanolone **12**, compound **7** – from epiandrosterone **13** by interaction with aromatic hydrazides, semicarbazide or methoxyamine, in ethanol medium and in the presence of a catalytic amount of acetic acid.

The structure of the synthesized compounds **1-7** was proved using ^{1}H-,^{13}C- NMR and mass spectra. In the ^{1}H NMR spectra of steroids **1-6**, singlet signals of the 18-CH_3, 19-CH_3, and 21-CH_3-groups were present, respectively, at δ 0.80-0.61 ppm, 0.93-0.74 ppm. and 2.09-1.73 ppm; protons of 18-CH_3, 19-CH_3-groups of hydrazone **7** – at δ 0.76, 0.87 ppm. Multiplet signals of 3α-protons from 3β-alcohols **1-7** had chemical shifts δ 3.34-3.33 ppm, protons from 3β-hydroxyl groups – at δ 4.49-4.41 ppm, protons Δ-16 of double C=C bonds of



Scheme. The transformation of ketone **11** to the corresponding steroids.

hydrazones **1, 2** – respectively at 6.30, 6.28 ppm. A singlet from the protons of the O-CH_3-group of oxime **6** was present at δ 3.66, while the protons of the NH_2- and NH-groups of semicarbazone **5** appeared at δ 6.17 and 8.82 ppm, respectively. Aromatic protons of hydrazones **1-4, 7** were noted in the range δ 8.34-7.00 ppm. The protons of the NHCO-groups were present as singlets in the range δ 10.78–10.29 ppm. The signals of the remaining protons corresponded to the proposed structures.

In the ^{13}C NMR spectra of 3β-alcohols **1-7**, downfield peaks from C-3 carbons were present in the region of δ 69.8-69.2 ppm, aromatic carbons in the range of δ 162.2-113.4 ppm. Signals of C=N bond were observed at δ 162.8-153.8 ppm, amide carbons NHCO for hydrazones **1-4** – in the region of δ 162.6-154.2 ppm. Steroids **1, 2** are characterized by signals from C-17 carbon, respectively, at δ 134.9, 135.0 ppm. and from C-16 at δ 148.8, 136.9 ppm. The signal from C-20 semicarbazone **5** was present at δ 149.8 ppm.

The molecular ions m/z $[M+H]^+$ corresponded to the brutto formulas of steroids **1-7**.

The cytotoxic effect of compounds **2-10** was investigated *in vitro* on cell cultures A-549 (lung cancer), DLD-1 (rectal cancer) and WS-1 (normal skin fibroblasts) using the resazurin recovery test and the Hoechst test according to the methods described in [12]. The first one, which reflects the metabolic activity of cells, makes it possible to evaluate the effect of the studied compounds on cell viability, while the second one is used for DNA-quantification and to calculate the number of living cells. Among all the compounds tested, only *p*-methyl- and *p*-methoxybenzoylhydrazone-5α-Pregnan-3β-ol-20-one **3, 4** may be of particular interest, since, unlike the others, they exhibit activity comparable to that of etoposide.

Experimental Part

^1H and ^{13}C NMR spectra were registered in DMSO on a spectrometer Avance 400 Bruker (400 MHz for ^1H and 100 MHz for ^{13}C). Internal standard – SiMe4. Mass spectra were obtained from an Agilent 1100 series HPLC-APCI MS (positive-ion mode) using an inertsil prep-ODS column (6.0 x 250 mm) and H_2O–CAN, 20:80 eluents. Melting points were determined on a NAGEMA apparatus. The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using benzene-aceton, 4:1 and benzene-methanol, 5:0.5. Chromatograms were detected by phosphomolybdic acid solution (10%) in EtOH followed by heating.

Oximes **6** were prepared by the literature method [4].

General method for synthesizing steroids 1-5, 7. A mixture of ketone **11, 12** or **13** was reacted with an equal amount by weight of steroid of the corresponding hydrazide and heated for 6-12 hours in ethanol in the presence of a catalytic amount of acetic acid. The reaction mixture was cooled to room temperature. The precipitate was filtered, washed with water and crystallized from ethanol.

5α-Pregn-16-en-3β-ol-20-one *p*-Nitrobenzoylhydrazone (1). Yield 75%, mp 248-250°C. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.77(3H, s, 18-CH_3), 0.93(3H, s, 19-CH_3), 2.09(3H, s, 21-CH_3), 3.33(1H, m, H-3), 4.49(1H, d, J=4.4, OH-3), 6.30(1H, m,H-16), 8.07(2H, d, J=8.2,H-Ar), 8.34(2H, d, J=8.3,H-Ar), 10.78(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.2 (C-3), 123.3(C-3′,5′), 129.2(C-2′,6′), 134.9(C-17), 139.9(C-1′), 148.8(C-16), 153.5(C-4′), 153.8 (C=N), 162.1(NHCO). LC-MS, m/z 480$[M+H]^+$. $C_{28}H_{37}N_3O_4$. MM 479.

5α-Pregn-16-en-3β-ol-20-one *m*-Bromobenzoylhydrazone (2). Yield 70%, mp 243-245^{0}C. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.80 (3H, s, CH_3-18), 0.93 (3H, s, CH_3-19), 2.01 (3H, s, CH_3-21), 3.34(1H, m, H-3), 4.49(1H, d, J=4.4, OH-3), 6.28(1H, m,H-16), 7.48(1H, m, ArH), 7.78(2H, m, ArH), 8.01(1H, s, ArH), 10.56(1H, s, NH-CO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.2 (C-3), 121.4(C-3′), 126.9(C-6′), 129.9(C-2′),

130.2(C-5′), 130.4(C-4′), 134.5(C-1′), 135.0(C-17), 136.9(C-16), 153.8(C=N), 154.2(NHCO). LC-MS, m/z 514 $[M+H]^+$. $C_{28}H_{37}BrN_2O_2$. MM 513.

5α-Pregnan-3β-ol-20-one *p*-Methylbenzoylhydrazone (3). Yield 69%, mp 249-251ºC. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.61(3H, s, 18-CH_3), 0.75(3H, s, 19-CH_3), 1.91(3H, s, 21-CH_3), 2.36(Ar-CH_3), 3.34(1H, m, H-3), 4.42(1H, d, J=4.4, OH-3), 7.28(2H, d, J=8.4,H-Ar), 7.74(2H, d, J=8.5,H-Ar), 10.30(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.2 (C-3), 127.5(C-3′,5′), 128.7(C-2′,6′), 131.3(C-1′), 141.1 (C-4′), 162.0(C=N), 162.6(NHCO). LC-MS, m/z 451 $[M+H]^+$. $C_{29}H_{42}N_2O_2$. MM 450.

5α-Pregnan-3β-ol-20-one *p*-Methoxybenzoylhydrazone (4). Yield 72%, mp 230-232ºC. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.73(3H, s, 18-CH_3), 0.88(3H, s, 19-CH_3), 1.96(3H, s, 21-CH_3), 3.33(1H, m, H-3), 3.81(3H, s, O-CH_3), 4.49(1H, s, OH-3), 7,00(2H, d, J=8.3, H-Ar), 7.84 (2H, d, J=8.4,H-Ar), 10.29(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.2(C-3), 113.4(C-3′,5′), 126.1(C-1′), 129.3(C-2′,6′), 161.5(NHCO), 162.2(C-4′), 162.8(C=N). LC-MS, m/z 467 $[M+H]^+$. $C_{29}H_{42}N_2O_3$. MM 466.

Semicarbazone-5α-Pregnan-3β-ol-20-one (5). Yield 77%, mp 258-260ºC. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, pp J/Hz): 0.63(3H, s, 18-CH_3), 0.74(3H, s, 19-CH_3), 1.75(3H, s, 21-CH_3), 3.34(1H, m, H-3), 4.49(1H, s, 3-OH), 6.17(1H, br.s, NH_2), 8.82(1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.8(C-3), 149.8(C-20), 158.0 (C=N). LC-MS, m/z 376 $[M+H]^+$. $C_{22}H_{37}N_3O_2$. MM 375.

20-O-Methyloxime-5α-Pregnan-3β-ol-20-one (6). Yield 89%, mp 146-148ºC. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.74(3H, s, 18-CH_3), 0.82(3H, s, 19-CH_3), 1.73(3H, s, 21-CH_3), 3.33(1H, m, H-3), 3.66(3H, s, O-CH_3), 4.41(1H, d, J=4.4, OH-3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.2(C-3), 159.3(C=N). LC-MS, m/z 348 $[M+H]^+$. $C_{22}H_{37}NO_2$. MM 347.

5α-Androstan-3β-ol-17-one *p*-Nitrobenzoylhydrazone (7). Yield 87%, mp 300-303ºC. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.76(3H, s, 18-CH_3), 0.87(3H, s, 19-CH_3), 2.39(1H, m, H-16), 2.59(1H, dd, J=19.5, 9.2, H-16), 3.34(1H, m, H-3), 4.47(1H, d, J=4.4, OH-3), 8.00(2H, d, J=8.2,H-Ar), 8.31(2H, d, J=8.3,H-Ar), 10.56 (1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 69.1(C-3), 123.3(C-3′,5′), 129.1(C-2′,6′), 140.0(C-1′), 149.0(C-4′), 161.9(NHCO), 175.6(C=N). LC-MS, m/z 454$[M+H]^+$. $C_{26}H_{35}N_3O_4$. MM 453.

ფარმაკოქიმია

ტიგოგენინიდან ახალი 5α-სტეროიდული ჰიდრაზონების სინთეზი

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(წარმოდგენილია აკადემიის წევრის ე. ქემერტელიძის მიერ)

პოტენციური ბიოლოგიურად აქტიური სტეროიდული ნაერთების სინთეზის მიზნით 5α-პრეგნენის, 5α-პრეგნანის და 5α-ანდროსტანის რიგის კეტონებისგან მიღებულია ზოგიერთი ახალი ჰიდრაზონი, სემიკარბაზონი და 20-O-მეთილოქსიმი. კეტონების - 5α-პრეგნ-16-ენ-3β-ოლ-20-ონის, 5α-პრეგნან-3β-ოლ-20-ონის და 5α-ანდროსტან-3β-ოლ-17-ონის კონდენსაციის რეაქციები სხვადასხვა სახის არილჰიდრაზიდებთან, O-მეთოქსიამინსა და სემიკარბაზიდთან ჩატარებულია ეთილის სპირტის არეში დუღილით, კატალიზატორად გამოყენებულია ძმარმჟავა. საწყისი კეტონები სინთეზირებულია სტეროიდული საპონინების აგლიკონის – ტიგოგენინის საფუძველზე, რომელიც წარმოადგენს სამამულო ნედლეულს 5α-რიგის სტეროიდების სინთეზისთვის. იგი გამოყოფილია საქართველოში ინტროდუცირებული მცენარე იუკა დიდებულიდან. ახალი სტეროიდული ნაერთების აღნაგობა დამტკიცებულია 1H, 13C-ბმრ და მას-სპექტრების მონაცემებით. ამ სტეროიდების და ჩვენ მიერ ადრე სინთეზირებული ჰიდრაზონების ციტოტოქსიკური აქტიურობა შესწავლილია in vitro ექსპერიმენტში, რეზაზურინის აღდგენის და Hoechst-ის ტესტის გამოყენებით. კვლევა ჩატარებულია კიბოს ზოგიერთი უჯრედული ხაზის – ფილტვის ადენოკარცინომის (A-549), სწორი ნაწლავის ადენოკარცინომის (DLD-1) და კანის ნორმალური ფიბრობლასტების (WS-1) მიმართ, ეტოპოზიდთან შედარებით. მიღებული შედეგი გვიჩვენებს, რომ შესწავლილი ნაერთებიდან მხოლოდ პ-მეთილ- და პ-მეთოქსიბენზოილ-5α-პრეგნან-3β-ოლ-20-ონი ამჟღავნებს ეტოპოზიდის დონის აქტიურობას.

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