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Pharmacochemistry

Modification of Pregnenolone to some Nitrogen-Containing Steroids

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(Presented by Academy Member Ether Kemertelidze)

Several novel pyrazolines and hydrazones have been synthesized as part of the search for biologically active steroids. From the starting compound 5α-pregn-16-en-3β-ol-20-one, 5α-androstan-3β-ol-17-one was obtained, which was modified with phenylacetic acid chloride to 3β-phenylacetoxy-5α-androstan-17-one. Condensation reaction of ketones – 5α-pregn-16-en-3β-ol-20-one and 3β-phenylacetoxy-5α-androstan-17-one with various arylhydrazines and arylhydrazides by boiling in ethanol in the presence of a catalytic amount of acetic acid the corresponding pyrazolines and esterified hydrazones were synthesized. The initial ketones were obtained from domestic raw material, the aglycone of steroid saponins – tigogenin. The structure of the obtained steroidal hydrazones and pyrazolines was proved using ^{1}H, ^{13}C-NMR and mass spectra data. In addition, the cytotoxic, antibacterial and antifungal activity of obtained steroids have been studied. Cytotoxic properties were assessed *in vitro* using the Resazurin reduction test on lung cancer (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 examined compounds only 2-pyridinocarbonylhydrazone-3β-phenylacetoxy-5α-androstan-17-one may be of particular interest since, unlike the others, it has activity equivalent to etoposide. It should be mentioned that two structural isomers of this hydrazone (nicotinoyl- and isonicotinoylhydrazones), which differ only in the location of the nitrogen atom in the heterocycle, lack this action. None of the investigated substances exhibit considerable antibacterial or antifungal efficacy. *© 2023 Bull. Georg. Natl. Acad. Sci.*

epiandrosterone, hydrazide, hydrazine, hydrazone, pyrazoline, 5α-steroids, cytotoxic

The structural modification of steroids is a hot topic in research, which requires significant synthetic efforts. Condensation of the steroid molecule ring with various nitrogen-containing arylderivatives can boost the biological activity of the modified steroids. Scientists are particularly interested in steroidal hydrazones and pyrazolines since they exhibit valuable pharmacological activities such as antifungal, antibacterial, antiproliferative, antituberculous, antiviral, and anticancer [1-3]. Steroid pyrazolines are also a viable framework for the development of novel medications due to their

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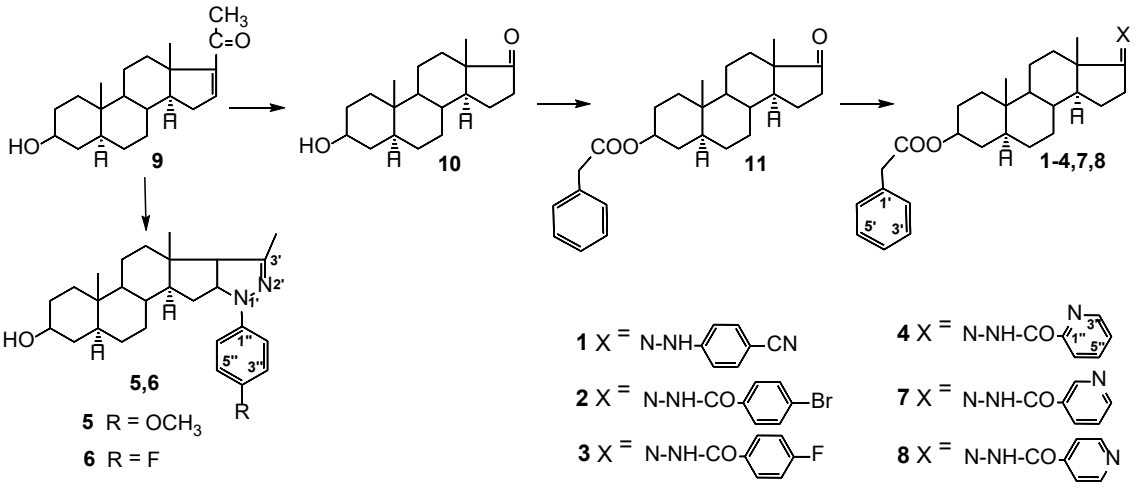
diverse chemical activity and wide spectrum of biological activity [4,5]. Some previously synthesized hydrazones and pyrazolines of 5α-steroids based on tigogenin also revealed high biological efficacy [6,7].

Novel hydrazones **1-4** and pyrazolines **5, 6** were synthesized during the ongoing search for biologically active 5α-steroids. The cytotoxic, antibacterial and antifungal activity of these and previously obtained [8,9] hydrazones: nicotinoyl- and isonicotinoylhydrazone of 3β-phenylacetoxy-5α-androstan-17-one **7,8** was studied. The starting pregnenolone **9** was converted into epiandrosterone **10**, which was modified with phenylacetic acid chloride to obtain 3β-phenylacetoxy-5α-androstan-17-one **11**. Hydrazones **1-4** were synthesized from this ketone **11**, and pyrazolines **5,**6 - from 5α-pregnenolone **9** by interaction with aromatic hydrazides or hydrazines in ethanol in the presence of a catalytic amount of acetic acid. It is known [10] that under such conditions, hydrazones of α,β-unsaturated ketones undergo intramolecular cyclocondensation to form pyrazolines. Cyclization proceeds at the moment of formation of hydrazones, which is facilitated by the presence of electron-donating substituents such as methoxy- and fluorophenyl groups, at the amine atom of hydrazine.

The structure of the synthesized compounds **1-6** 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- groups were present respectively, at δ 0.82-0.90 ppm and 0.90-1.03 ppm., singlet signals of the 21-CH_3-groups of steroids **5,6** - at δ 2.07-2.08 ppm. Multiplet signals of 3α-protons from 3β-esters **1-4** had chemical shifts δ 4.61-4.75 ppm, protons from 3β-hydroxyl groups of pyrazoline **5,6** – at δ 3.61-3.62 ppm. The signals of hydrogen atoms at C-17 of pyrazolines **5,6** were noted at δ 3.20 and 3.22 ppm, C-16 protons – at 4.44 and 4.45 ppm, respectively. Aromatic protons of hydrazones **1-4** were noted in the range of δ 7.09-8.57 ppm, pyrazolines **5,6** - at δ 6.87-7.09 ppm. The protons of the NH group of hydrazone **1** were present at δ 9.23ppm, signals of the NHCO-groups from steroids **2-4** observed as singlets in the range δ 8.36-10.44 ppm. The signals of the remaining protons corresponded to the proposed structures.

In the ^{13}C NMR spectra, the C-3 signals of 3β-esters **1-4** were present in the region of 73.8-74.1 ppm, the higher-field signals of 3β-alcohols **5,6** – at δ 71.1 and 71.2 ppm, aromatic carbons were in the range δ 98.5-166.0 ppm. Peaks of the C=N bond of hydrazones **1-4** were observed in the range of δ 164.3-171.2 ppm, for pyrazolines **5,6** - at δ 152.3



Scheme. The transformation of ketones **9,11** to the corresponding steroids **1-8.**

and 149.5 ppm, respectively, amide NHCO carbons – in the region of δ 159.7 -162.2 ppm. Steroids **5,6** are characterized by signals from C-16 carbon – at δ 65.0 and 64.7 ppm and C-17 - at δ 66.5 and 66.6 ppm, for nitrile **1** from the C≡N bond – at 112.5 ppm. Signals of O-C=O-carbon of steroids **1-4** were presented in the range of δ 171.1-172.5 ppm.

The molecular ions m/z $[M+H]^+$ corresponded to the brutto formulas of steroids **1-6**.

The cytotoxic effect of compounds **1-8** was studied *in vitro* on cell cultures A-549 (lung cancer), DLD-1 (rectal cancer) and WS-1 (normal skin fibroblasts) using the resazurin reduction test and the Hoechst test according to the method [11]. 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 to calculate the number of living cells. Of all the tested compounds, only 2-pyridinocarbonylhydrazone 3β-phenylacetoxy-5α-androstan-17-one (**4**) may be of particular interest, since, unlike the others, it exhibits an activity comparable to that of etoposide (without specific activity). It should be noted that of the three structural isomers (**4,7,8**) that differ only in the position of the nitrogen atom in the heterocycle, only one (hydrazone **4**) is characterized by notable activity. Antibacterial and antifungal efficacy testing found that none of the investigated compounds (**1-8)** were active when compared to gentamicin and amphotericin B, respectively.

Experimental Part

^1H and ^{13}C NMR spectra were registered in DMSO and $CDCl_3$, 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.

General method for synthesizing steroids 1-6. A solution of 1 mmol of ketone **9** or **11** in ethanol was mixed with 1.20 mmol of the corresponding hydrazine or hydrazide and boiled for 6-14 hours in the presence of a catalytic amount of acetic acid. The precipitate formed after cooling the mixture to room temperature was filtered out, washed with water, dried, and crystallized from methanol.

3β-Phenylacetoxy-5α-androstan-17-one *p*-cyanophenylhydrazone (1). Yield 60%, mp 194-196°C. ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.82(6H, s, 18-CH_3, 19-CH_3), 2.31(1H, m, H-16), 2.45(1H, m, H-16),3.62(2H, s,$\underline{CH_2}C_6H_5$), 4.61(1H, m, H-3), 7.09(2H, d, J=8.3,H-Ar), 7.25-7.34(5H, m, C_6H_5), 7.53(2H, d, J=8.2,H-Ar), 9.23(1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 73.8(C-3), 98.5(C-4$^{//}$), 112.5(C≡N), 120.8(C-4$^{/}$), 127.2(C-2$^{//}$,6$^{//}$), 128.8(C-3$^{//}$,5$^{//}$), 129.8(C-3$^{/}$,5$^{/}$), 133.8(C-2$^{/}$,6$^{/}$), 135.0(C-1$^{/}$), 150.4(C-1$^{//}$), 164.3(C=N), 171.1(O-C=O). LC-MS m/z 524$[M+H]^+$. $C_{34}H_{41}N_3O_2$. MM 523.

3β-Phenylacetoxy-5α-androstan-17-one *p*-bromobenzoylhydrazone (2). Yield 75%, mp 214-216°C. ^1H NMR spectrum (400 MHz, $CDCl_3$,δ, ppm, J/Hz): 0.89(3H, s, 18-CH_3), 1.03(3H, s, 19-CH_3), 2.31(2H, m, H-16), 3.62(2H, s,$\underline{CH_2}C_6H_5$), 4.75(1H, m, H-3), 7.30-7.36 (5H, m, C_6H_5), 7.61-7.91(4H, m, H-Ar), 8.36(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, $CDCl_3$,δ, ppm): 74.1(C-3), 127.0-134.4(12C-Ar), 162.2(NHCO), 166.3(C=N), 171.2(O-C=O). LC-MS m/z 606$[M+H]^+$. $C_{34}H_{41}BrN_2O_3$. MM 605.

3β-Phenylacetoxy-5α-androstan-17-one *p*-fluorobenzoylhydrazone (3). Yield 68%, mp 193-195°C. ^1H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.89(3H, s, 18-CH_3), 0.99(3H, s, 19-CH_3), 2.31(1H, m, H-16), 2.49(1H, m, H-16), 3.62(2H, s, $\underline{CH_2}C_6H_5$), 4.75(1H, m, H-3), 7.15(2H, m, H-Ar), 7.29-7.38(5H, m, C_6H_5), 7.96(2H, m, H-

Ar), 8.38(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 74.1(C-3), 116.1-134.4(11C-Ar), 162.2(NHCO), 166.0(C-4$^{//}$), 166.5(C=N), 171.2(O-C=O). LC-MS m/z 545[M+H]$^+$. $C_{34}H_{41}FN_2O_3$. MM 544.

3β-Phenylacetoxy-5α-androstan-17-one 2-pyridinecarbohydrazone (4). Yield 71%, mp 195-196^{o}C. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.90(3H, s, 18-CH_3), 1.00(3H, s, 19-CH_3), 2.45(1H, m, H-16), 2.66(1H, m, H-16), 3.62(2H, s, $\underline{CH_2}C_6H_5$), 4.75(1H, m, H-3), 7.29-7.37(5H, m, C_6H_5), 7.48(1H, m, H-Ar), 7.90(2H, t, J=8.4,H-Ar), 8.31(1H, d, J=7.8, H-Ar), 8.57(1H, d, J=4.3,H-Ar), 10.44(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 74.1 (C-3), 122.8(C-6$^{//}$), 126.4(C-4$^{//}$), 127.0(C-4$^{/}$), 128.5(C-3$^{/}$,5$^{/}$), 129.2(C-2$^{/}$,6$^{/}$), 134.4(C-1$^{/}$), 137.5(C-5$^{//}$), 148.0(C-3$^{//}$), 149.8(C-1$^{//}$), 159.7(NHCO), 171.2(C=N), 172.5(O-C=O). LC-MS m/z 528[M+H]$^+$. $C_{33}H_{41}N_3O_3$. MM 527.

3β-Hydroxy-1$^{/}$-*p*-methoxyphenyl-3$^{/}$-methyl-5α-androstano[17,16-d]pyrazoline (5). Yield 80%, mp 255-257^{o}C. ^{1}H NMR spectrum (400 MHz, $CDCl_3$,δ, ppm, J/Hz): 0.85 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 2.07 (3H, s, 21-CH_3), 3.20 (1H, d, *J*=10.0, H-17), 3.61(1H, m, H-3), 3.80(3H,s,O-CH_3), 4.44(1H, dd, J=10.1, 5.6, H-16), 6.87 (2H, d, J=8.3, H-Ar), 6.96 (2H, d, J=8.3, H-Ar). ^{13}C NMR spectrum (100 MHz, $CDCl_3$,δ, ppm): 55.9(O-CH_3), 65.0(C-16), 66.5(C-17), 71.1(C-3), 112.9(C-2$^{//}$,6$^{//}$), 114.7(C-3$^{//}$,5$^{//}$), 140.1(C-1$^{//}$), 148.8(C-4$^{//}$), 152.3 (C=N). LC-MS m/z 437[M+H]$^+$, $C_{28}H_{40}N_2O_2$. MM 436.

3β-Hydroxy-1$^{/}$-*n*-fluorophenyl-3$^{/}$-methyl-5α-androstano[17,16-d]pyrazoline (6). Yield 58%, mp 224-226^{o}C. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.86 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 2.08 (3H, s, 21-CH_3), 3.22 (1H, d, *J* = 10.1, H-17), 3.62(1H, m, H-3), 4.45(1H, dd, J=10.1, 5.9, H-16), 6.91-7.09 (4H, m, H-Ar). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 64.7(C-16), 66.6(C-17), 71.2(C-3), 112.6(C-2$^{//}$,6$^{//}$), 115.5(C-3$^{//}$,5$^{//}$), 141.9(C-1$^{//}$), 149.5(C=N), 155.2(C-4$^{//}$). LC-MS m/z 425[M+H]$^+$, $C_{27}H_{37}N_2FO$. MM 424.

ფარმაკოქიმია

პრეგნენოლონის მოდიფიცირებით ზოგიერთი აზოტშემცველი სტეროიდის მიღება

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(წარმოდგენილია აკადემიის წევრის ე. ქემერტელიძის მიერ)

ბიოლოგიურად აქტიური სტეროიდული ნაერთების სინთეზის მიზნით მიღებულია ზოგიერთი ახალი პირაზოლინი და ჰიდრაზონი. საწყისი ნაერთიდან - 5α-პრეგნ-16-ენ-3β-ოლ-20-ონი სინთეზირებულია 5α-ანდროსტან-3β-ოლ-17-ონი, რომელიც ფენილმმარმჟავას ქლორანჰიდრიდის გამოყენებით გარდავქმენით 3β-ფენილაცეტოქსი-5α-ანდროსტან-3β-ოლ-17-ონად. კეტონების - 5α-პრეგნ-16-ენ-3β-ოლ-20-ონის და 3β-ფენილაცეტოქსი-5α-ანდროსტან-3β-ოლ-17-ონის კონდენსაციის რეაქცია სხვადასხვა სახის არილჰიდრაზინსა და არილჰიდრაზიდთან ჩატარებულია ეთილის სპირტის არეში დუღილით, ძმარმჟავას კატალიზური რაოდენობის გამოყენებით სინთეზირებულია პირაზოლინები და ესთერიფიცირებული ჰიდრაზონები. საწყისი კეტონები მიღებულია სამამულო ნედლეულის, სტეროიდული საპონინების აგლიკონის - ტიგოგენინის საფუძველზე. ახალი სტეროიდული ნაერთების აღნაგობა დამტკიცებულია ^{1}H, 13C-ბმრ და მას-სპექტრების მონაცემებით. შესწავლილია ამ სტეროიდების ციტოტოქსიკური, ანტიბაქტერიული და ანტიმიკოზური აქტიურობა. ციტოტოკსიკური აქტიურობა შესწავლილია ***in vitro*** ექსპერიმენტში, რეზაზურინის აღდგენის ტესტის გამოყენებით. კვლევა ჩატარებულია კიბოს ზოგიერთი უჯრედული ხაზის - ფილტვის კარცინომის (A-549), სწორი ნაწლავის კიბოს (DLD-1) და კანის ნორმალური ფიბრობლასტების (WS-1) მიმართ, ეტოპოზიტთან შედარებით. მიღებული შედეგები გვიჩვენებს, რომ შესწავლილი ნაერთებიდან მხოლოდ 3β-ფენილაცეტოქსი-5α-ანდროსტან-17-ონის 2-პირიდინოკარბონილჰიდრაზონი სხვა ნაერთებისგან განსხვავებით, ამჟღავნებს ეტოპოზიტის დონის აქტიურობას. აღსანიშნავია, რომ აღნიშნული ჰიდრაზონის სხვა ორი სტრუქტურული იზომერი (ნიკოტინოილ- და იზონიკოტინოილ ჰიდრაზონები), რომელიც განსხვავდება მხოლოდ აზოტის ატომის მდებარეობით ჰეტეროციკლში, არ ამჟღავნებს მსგავს აქტიურობას. არცერთი ტესტირებული ნაერთი არ ავლენს მნიშვნელოვან ანტიბაქტერიულ და ანტიმიკოზურ მოქმედებას.

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