საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 15, №3, 2021
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 15, no. 3, 2021

Pharmacochemistry

Synthesis of Some Novel Nitrogen-Containing 5α-Steroids Based on Tigogenin

Nanuli Nadaraia[*], Nana Barbakadze[*], Karen Mulkijanyan[*], Vakhtang Mshvildadze[**], Jean Legault[**]

[*]*Iovel Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Georgia*
[**]*LASEVE, Universite Quebec a Chicoutimi, Chicoutimi, Qc,Canada*

(Presented by Academy Member Ether Kemertelidze)

A number of nitrogen-containing compounds were obtained using a condensation reaction catalyzed by acetic acid to investigate structure-chemical reactivity and structure-biological activity relationships in the search for potential biologically active steroids within ketones of the 5α-pregnane and 5α-androstane series. Novel steroidal hydrazones were synthesized from 5α-pregnan-3β-ol-20-one and 5α-pregna-9(11),16-dien-3α-ol-20-one, which, in turn, were obtained by a multistep transformation of 3β-acetoxy-5α-pregn-16-en-20-one. All of the starting steroid ketones were synthesized on the basis of a convenient domestic raw material – tigogenin, isolated from the *Yucca gloriosa* plant introduced in Georgia. Acetic acid catalyzed condensation reaction was carried out in ethanol using various reagents with pharmacophoric features – arylhydrazides, arylhydrazines, hydroxylamine, and semicarbazide. The structure of the newly obtained steroids was confirmed by ^{1}H, ^{13}C NMR, mass spectra and investigation of their biological activity is in process. The cytotoxic and antiviral activity of the previously synthesized steroid oximes, amines and hydrazones was assessed. *© 2021 Bull. Georg. Natl. Acad. Sci.*

Pregnenolone, epiandrosterone, hydrazine, hydrazone, 5α-steroids, antiviral activity, cytotoxic activity

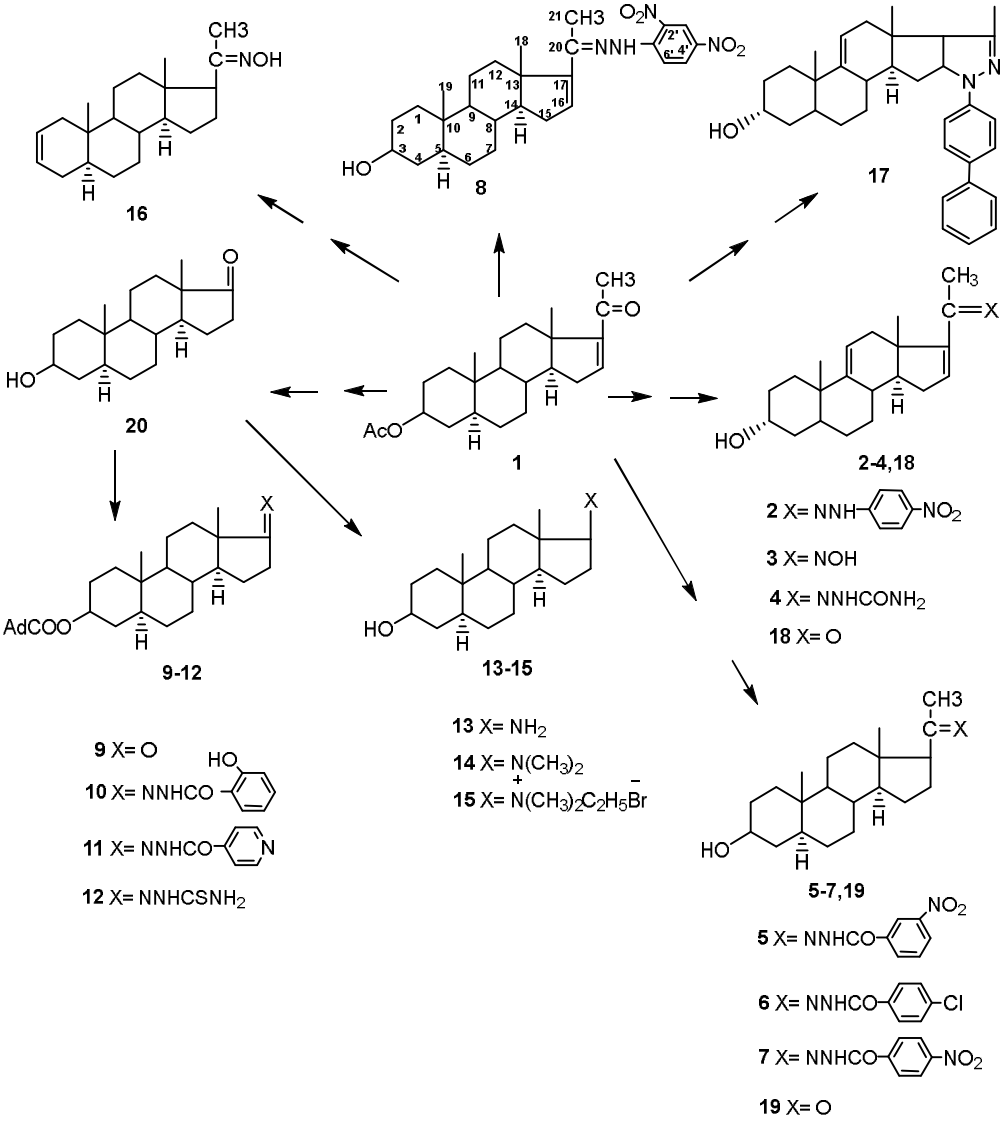
Screening of nitrogen-containing steroids for the biological activity makes it possible to identify leaders, precursors of drugs, and create new libraries of potential therapeutic agents [1, 2].

Synthetic 17-aminosteroids of the androstane series, which are widely used as key intermediates in the synthesis of various biologically active steroid derivatives, themselves have significant biological activity, including antitumor and antiarrhythmic activity [3,4]. Steroid oximes [5,6] and hydrazones [7,8] are considered pharmacologically active, as well.

A number of new steroids **2-8** were synthesized in the continued search for new bioactive steroids [9] based on a product of tigogenin conversion - 5α-pregnenolone acetate **1**. The work also provides data on the antiviral (for steroids **9**-**16**) and cytotoxic (for steroids **2,3,17**) activity of nitrogen-containing steroids synthesized by us earlier: 3β-(1-adamantoate)-5α-androstan-17-one **9**,

salicyloylhydrazone 3β-(1-adamantoate)-5α-androstan-17-one **10**, isonicotinoylhydrazone 3β-(1-adamantoate)-5α-androstan-17-one **11**, thiosemicarbazone 3β- (1-adamantoate)-5α-androstan-17-one **12**, 17β-amino-5α-androstan-3β-ol **13**, 17β-(N, N-dimethylamino-5α-androstan-3β-ol **14**, bromide N-(3β-hydroxy-5α-androstan-17β-yl) dimethylethylammonium **15**, 20-hydroxyimino-5α-pregn-2-ene**16**, 3α-Hydroxy-1′-p-phenylphenyl-3′-methyl-5α-androst-9(11)-eno[17,16-d]pyrazoline **17.**

Steroids **2-4, 17** were synthesized from ketone **18**, 3α-hydroxy-5α-pregna-(9)11,16-dien-20-one **18** was obtained by subsequent conversion of 5α-pregnenolone acetate **1** according to the method [10,11]. Hydrazones **5-7** were synthesized from pregnanolone **19**, which, in turn, was obtained by catalytic hydrogenation of the starting ketone **1** according to the method [9]. The same ketone was used to synthesize hydrazone **8**, oxime **16** and epiandrosterone **20**, which was converted into ester **9**, hydrazones **10-12** and amino derivatives **13-15** [12-14].



Scheme 1. The transformation of ketone **1** to the corresponding steroids.

The structure of the synthesized compounds **2-8** was proved by ^{1}H-, ^{13}C NMR and mass spectra. In the ^{1}H NMR spectra of steroids **2-4**, singlet signals of 18-CH_3, 19-CH_3 and 21-CH_3-groups were present, respectively, at δ 0.81-0.75 ppm, 0.96-0.82 ppm and 2.06-1.91 ppm. 3β-Protons of 3α-alcohols **2-4** had the values of chemical shifts δ 4.19-4.06 ppm in the form of broadened singlets, protons from 3α-hydroxyl groups - at δ 3.82-3.80 ppm, Δ-9(11)- and Δ-16 protons of double C = C bonds – at δ 5.36-5.30 ppm, respectively and 6.14-6.05 ppm. A broadened singlet from the C = N-OH proton of oxime **3** was present at δ 8.26, while the protons of the NH_2 and NH groups of semicarbazone **4** appear at δ 6.30 and 9.10 ppm, respectively. Aromatic protons and signals of NH-groups of hydrazone **2** were noted in the range of δ 8.40-7.18 ppm and at δ 9.93 ppm. In the ^{1}H NMR spectra of steroids **5-8** signals of 18-CH_3, 19-CH_3 and 21-CH_3-groups were present in the form of singlets at δ 0.88-0.58 ppm, 1.05-0.76 ppm, respectively and 2.19-1.92 ppm. Multiple 3α-protons from 3β-alcohols were noted at δ 3.33 ppm. and at 3.60 ppm for hydrazone **8** (in $CDCl_3$). Aromatic protons appeared in the range of 9.14 - 7.56 ppm, singlet protons of NHCO-groups of compounds **5-7** – in the range of δ 10.79-10.48 ppm and NH-proton of hydrazone **8** – at δ 11.10 ppm in the form of br. singlet. The signals of the remaining protons corresponded to the proposed structures.

In the ^{13}C NMR spectra of 3α-alcohols **2-4** signals from C-3 were noted at δ 66.7-64.0 ppm, peaks of carbons C-9 – at 151.9-145.7 ppm and C-11 at 115.8-111.7 ppm. Signals from C-16 were observed in the range 152.6-138.8 ppm, C-17 – 132.6-131.7 ppm, C = N bonds – δ 157.0-153.9 ppm, aromatic carbons of hydrazone **2** – in the range δ 145.5-123.4 ppm. In the ^{13}C NMR spectra of epimeric 3β-alcohols **5-7** (in DMSO), lower-field peaks from C-3 carbons were present in the δ 69.8-68.4 ppm range, aromatic carbons in the δ 149.5-121.5 ppm range. Signals of the C = N bond were observed at δ 167.3-164.3 ppm, amide NHCO carbons – in the range of δ 162.3-159.7 ppm. For hydrazone **8** (in CDCl3), the following signals are characteristic: from carbon C-3 – at δ 71.4 ppm., for C = C aromatic carbons in the area – δ 151.6-117.2 ppm, from C-17 and C-16 – at δ 137.6, 145.4 ppm and from C = N bond –154.0 ppm. Mass spectra confirmed the brutto formulas of compounds **2-8**.

Cytotoxic activity of steroids **2,3** and **17** was investigated *in vitro* on cancer cultures (A-549 - lung cancer, DLD-1 - rectal cancer and WS-1 - normal skin fibroblasts) using the resazurin reduction test. None of the compounds revealed cytotoxic activity.

Compounds **9-16** did not express notable antiviral activity when screened against the following viral strains: Adenovirus (cell culture A-549, strain 65089 / Chicago), Flu A (H1N1) virus (cell culture MDCK, strain California / 07/2009), Flu A (H3N2) virus (MDCK cell culture, strain Brisbane / 10/2007), Flu A (H5N1) virus (MDCK cell culture, Vietnam / 1203 / 2004H strain), Flu B virus (cell culture MDCK, strain Florida / 4/2006), PIV virus (cell culture MA-104, strain 14702), RSV A virus (cell culture MA-104, strain A2), Rhinovirus (cell culture Hela Ohio-1, strain HGP), Measlesvirus (Vero 76 cell culture, Chicago strain) and Sarsvirus (Vero 76 cell culture, Urbani strain).

Experimental Part

^{1}H and ^{13}C NMR spectra were recorded in DMSO and $CDCI_3$ on an Avance 400 Bruker spectrometer (400.13 MHz for 1H and 100.61 MHz for 13C). Internal standard – $SiMe_4$. Mass spectra were obtained on HPLC-APCIMS (positive mode) – Agilent 1100 series, column 6.0 x 250 mm Inertsilprep-ODS, with steroid elution in the H_2O-ACN system, 20:80. Melting points were determined on a NAGEMA unit. The course of the reaction and the purity of the synthesized compounds were monitored by TLC on Silufol-UV-254 plates in benzene-acetone, 5: 1 and benzene-methanol, 5: 0.5 systems. Chromatograms

were developed with a 10% solution of phosphoric-molybdic acid in ethanol followed by heating.

Oximes **3, 16** were obtained by the method described in [12].

General procedure for the synthesis of steroids 2-8. A mixture of ketone **1, 18** or **19** was reacted with an equal weight of the corresponding hydrazide or hydrazine hydrochloride and refluxed 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 that formed was filtered off, washed sequentially with water and diethyl ether, dried, and crystallized from methanol.

5α-Pregna-9(11),16-dien-3α-ol-20-one *p*-Nitrophenylhydrazone (2). Yield 69%, mp 220-222°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.78 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 2.06(3H, s, 21-CH_3), 3.82 (1H, s, 3-OH), 4.19(1H, br.s, H-3), 5.36(1H,m, H-11), 6.14(1H, s, H-16), 7.18-8.40 (4H, H-Ar), 9.93 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 11.4, 15.2, 17.0, 17.5, 20.3, 30.0, 30.4, 30.6, 31.2, 31.8, 33.2, 36.1, 46.8, 54.2, 57.5, 64.4(C-3), 111.7(C-11), 123.4(C-4′), 125.4(2C-2′,6′), 126.3(2C-3′,5′), 132.4(C-17), 138.3(C-16), 145.5(C-1′), 151.9(C-9), 153.9(C=N). LC-MS, m/z 450 $[M+H]^+$. $C_{27}H_{35}N_3O_3$. MM 449.

20-Hydroxyimino-5α-Pregna-9(11),16-dien-3α-ol-20-one (3). Yield 72%, mp 178-180°C. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm J/Hz): 0.81 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 2.00(3H, s, 21-CH_3), 4.06(1H, br.s, H-3), 5.33(1H,m, H-11), 6.05(1H, s, H-16), 8.26(1H, br.s, C=N-OH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm J/Hz): 11.1, 15.7, 17.0, 20.8, 28.5, 29.1, 31.9, 32.1, 33.9, 35.8, 36.3, 39.3, 46.9, 54.7, 57.1, 66.7(C-3), 115.8(C-11), 132.6(C-17), 147.9(C-9), 152.6(C-16), 154.9(C=N). LC-MS, m/z 330$[M+H]^+$. $C_{21}H_{31}NO_2$. MM 329.

Semicarbazone-5α-Pregna-9(11),16-dien-3α-ol-20-one (4). Yield 70%, mp>250°C(dec.). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.75 (3H, s, 18-CH_3), 0.82 (3H, s, 19-CH_3), 1.91((3H, s, 21-CH_3), 3.80(1H, s, 3-OH), 4.16(1H, br.s, H-3), 5.30(1H,m, H-11), 6.07(1H, s, H-16), 6.30(1H, br.s, NH_2), 9.10(1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 11.0, 13.3, 16.0, 19.9, 21.1 28.1, 28.5, 31.5, 33.4, 35.5, 35.7, 35.9, 46.3, 54.2, 56.8, 64.0(C-3), 115.1(C-11), 131.7(C-17), 142.9(C-16), 145.7(C-9), 153.0(C-20), 157.0(C=N). LC-MS m/z, 372 $[M+H]^+$. $C_{22}H_{33}N_3O_2$. MM 371.

5α-Pregnane-3β-ol-20-one *m*-Nitrobenzoylhydrazone (5). Yield 67%, mp 241-243°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.76(3H, cs 18-CH_3), 0.89(3H, s, 19-CH_3), 1.96(3H, s, 21-CH_3), 3.33(1H, m, H-3), 4.41(1H, d, J=4.4, OH-3), 7.79(1H, t, J=8.2,H-Ar), 8.31(1H, d, J=7.1,H-Ar), 8.40(1H, d, J=7.3, H-Ar), 8.66(1H, s, H-Ar), 10.79(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm):11.2, 19.8, 19.9, 23.8, 24.7, 27.5, 30.4, 31.3, 33.7, 34.2, 34.5, 35.7, 37.3, 43.3, 43.4, 44.1, 48.7, 52.6, 56.5, 68.4 (C-3), 121.5 (C-2′), 124.9 (C-4′), 129.5 (C-5′), 133.2 (C-6′), 135.0(C-1′), 146.8(C-3′), 159.7(NHC=O), 167.3(C=N). LC-MS, m/z 482$[M+H]^+$. $C_{28}H_{39}N_3O_4$. MM 481.

5α-Pregnane-3β-ol-20-one *p*-Chlorobenzoylhydrazone (6). Yield 75%, mp 252- 254°C. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.58(3H, s, 18-CH_3), 0.76(3H, s, 19-CH_3), 1.92(3H, s, 21-CH_3), 3.33(1H, m, H-3), 4.42(1H, d, J=4.4, OH-3), 7.56(2H, d, J=8.4, H-Ar), 7.84(2H, d, J=8.5, H-Ar), 10.48(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm):12.6, 13.7, 19.4, 21.3, 23.1, 24.4, 28.8, 31.8, 32.1 35.6, 35.7, 37.1, 44.0, 44.3, 44.9, 54.3, 56.2, 59.3, 63.2, 69.8(C-3), 128.8(C-3′,5′), 130.1(C-2′,6′), 134.5(C-1′), 136.7(C-4′),162.3(NHCO), 164.3(C=N). LC-MS, m/z 471.5$[M+H]^+$. $C_{28}H_{39}N_2ClO_2$. MM 470.5.

5α-Pregnane-3β-ol-20-one *p*-Nitrobenzoylhydrazone (7). Yield 80%, mp 237-238ºC. NMR spectrum (400 MHz, DMSO-d_6, δ, ppm J/Hz): 0.76(3H, s, 18-CH_3), 0.89(3H, s, 19-CH_3), 1.96(3H, s, 21-CH_3), 3.33(1H, m, H-3), 4.42(1H, d, J=4.4, OH-3), 8.07(2H, d, J=8.2,H-Ar), 8.33(2H, d, J=8.3,H-Ar), 10.75(1H, s, NHCO). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm):12.6, 13.7, 19.4, 21.3, 23.1, 24.4, 28.9, 31.8, 32.1 35.6, 35.7, 37.1, 44.3, 44.9, 45.5, 54.3, 56.2, 57.9, 59.3, 69.8(C-3), 123.9(C-3′,5′), 129.6(C-2′,6′), 140.5(C-1′), 149.5(C-4′),161.8(NHCO), 165.3(C=N). LC-MS m/z 482$[M+H]^+$, $C_{28}H_{39}N_3O_4$ MM 481.

5α-Pregn-16-en-3β-ol-20-one 2,4-Dinitrophenylhydrazone (8). Yield 78%, mp 253-255ºC. NMR spectrum (400 MHz, $CDCl_3$, δ, ppm J/Hz): 0.88 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 2.19(3H, s, 21-CH_3), 3.60(1H, m, H-3), 6.29(1H, m, H-16), 7.87(1H, d, J=9.6, H-Ar), 8.35(1H, dd, J=9.6, 2.5, H-Ar), 9.14(1H, d, J= 2.5, H-Ar), 11.10(1H, br.s, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$,δ, ppm): 12.4, 13.5, 16.3, 21.5, 28.7, 31.6, 31.9, 32.0, 33.9, 35.8, 36.1, 36.9, 38.2, 45.2, 47.2, 54.8, 56.9, 71.4 (C-3), 117.2(C-6′), 124.6 (C-3′), 129.9 (C-2′), 130.7(C-5′), 137.6 (C-17), 138.4 (C-4/), 145.4(C-16), 151.6 (C-1′), 154.0 (C=N). LC-MS, m/z $[M+H]^+$ 497. $C_{27}H_{36}N_4O_5$.. MM 496.

The authors are grateful to Dr. Cecil D. Kwong (Southem Research Institute-USA) for assistance in determining the antiviral activity of the synthesized compounds.

ფარმაკოქიმია

ტიგოგენინის ბაზაზე ზოგიერთი ახალი აზოტშემცველი 5α-სტეროიდის სინთეზი

ნ. ნადარაია*, ნ. ბარბაქაძე*, კ. მულკიჯანიანი*, ვ. მშვილდაძე**, ჟ. ლეგო**

*თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, იოველ ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, თბილისი, საქართველო
**LASEVE, Universite Quebec a Chicoutimi,Chicoutimi, Qc, კანადა

(წარმოდგენილია აკადემიის წევრის ე. ქემერტელიძის მიერ)

პოტენციური ბიოლოგიურად აქტიური სტეროიდული ნაერთების სინთეზის, ქიმიური სტრუქტურა-რეაქციის უნარიანობასა და ქიმიური სტრუქტურა-ბიოლოგიურ აქტიურობას შორის კავშირის შესწავლის მიზნით, 5α-პრეგნანის და 5α-ანდროსტანის რიგის კეტონებისგან მიღებულია ზოგიერთი ახალი აზოტშემცველი ნაერთი ძმარმჟავათი კატალიზებული კონდენსაციის რეაქციის გამოყენებით. ახალი სტეროიდული ჰიდრაზონები სინთეზირებულია 5α-პრეგნან-3β-ოლ-20-ონის და 5α-პრეგნა-9(11),16-დიენ-3α-ოლ-20-ონისგან, რომლებიც თავის მხრივ მიღებულია 3β-აცეტოქსი-5α-პრეგნ-16-ენ-20-ონის მრავალ-საფეხურიანი გარდაქმნით. ყველა საწყისი კეტონი სინთეზირებულია სტეროიდების მისაღებად ხელსაყრელი, სამამულო ნედლეულის - ტიგოგენინის საფუძველზე, რომელიც გამოყოფილია საქართველოში ინტროდუცირებული მცენარის იუკა დიდებულიდან. ძმარმჟავათი კატალიზებული კონდენსაციის რეაქცია ჩატარებულია ეთილის სპირტის არეში სხვადასხვა ფარმაკოფორული რეაგენტის – არილჰიდრაზიდების, არილჰიდრაზინების, ჰიდროქსილამინის და სემიკარბაზიდის გამოყენებით. ახალი სტეროიდული ნაერთების აღნაგობა დადასტურებულია ^{1}H,^{13}C-ბმრ და მასსპექტრების მონაცემებით. ეს ნაერთები გადაცემულია ბიოლოგიური აქტიურობის შესაფასებლად. შესწავლილია ჩვენ მიერ ადრე სინთეზირებული სტეროიდული ოქსიმების, ამინების და ჰიდრაზონების ციტოტოქსიკური და ანტივირუსული აქტიურობა.

REFERENCES

1. Jegham H., Maltais R., Roy J., Doillon C., Poirier D. (2012) Biological evaluation of a new family of aminosteroids that display a selective toxicity for various malignant cell lines. *Anticancer Drugs*, **23**,8: 803-814.
2. Verma G., Marella A., Shaquiquzzaman M., Akhtar M., Ali M. R., Alam M. M. (2014) A review exploring biological activities of hydrazones. *Journal of Pharmacy and Bioallied Sciences,* **6,** 2: 69-80.
3. Merlani M.I., Amiranashvili L.S., Mulkijanyan K.G., Kemertelidze E.P.(2006) Synthesis and biological activity of certain amino-derivatives of 5α-steroids. *Chem. Nat.Compd.,* **42**:322-324.
4. Nadaraia N.Sh., Amiranashvili L.Sh., Merlani M.I. (2016) Structure-activity relationship of epimeric 3,17-substituted 5α-androstane aminoalcohols. *Chemistry of Natural Compounds,* **52**:961-962.
5. Canário C., Silvestre S., Falcão A., Alves G. (2018) Steroidal oximes: useful compounds with antitumor activities. *Current Medicinal Chemistry,* **25:**1-27.
6. Banday A. H., Akram S.M.M., Shameem Sh. A.(2014) Benzylidine pregnenolones and their oximes as potential anticancer agents: synthesis and biological evaluation. *Steroids,* **84**:64-69.
7. Cui J.G., Fan L., Huang Y., Xin Y., Zhou A. (2009) Synthesis and evaluation of some steroidal oximes as cytotoxic agents: structure/activity studies (II). *Steroids,* **74**: 989-995.
8. Cui J., Liu L., Zhao D., Gan Ch., Huang X., Xiao Q., Qi B., Yang L., Huang Y. (2015) Synthesis, characterization and antitumor activities of some steroidal derivatives with side chain of 17-hydrazone aromatic heterocycle. *Steroids,* **95**:32-38.
9. Nadaraia N.Sh., Kakhabrishvili M.L., Barbakadze N.N., Mshvildadze V.D., Mulkijanyan K.G,Pichette A. (2021) Synthesis and cytotoxicity of 5α-pregnan-3β-ol-20-one hydrazones. *Chemistry of Natural Compounds,* **57:**395-397.
10. Nadaraia N.Sh., Kakhabrishvili M.L., Barbakadze N.N., Mshvildadze V.D., Silla B., Legault J., Pichette A. (2018) Synthesis and biological activity of steroidal tigogenin hydrazones and pyrazolines. *Chemistry of Natural Compounds,* **54:**926-929.
11. Nadaraia N.Sh., Amiranashvili L. Sh., Merlani M., Kakhabrishvili M. L., Barbakadze N. N., Geronikaki A., Petrou A., Poroikov V., Ciric A., Glamoclija J., Sokovic M. (2019) Novel antimicrobial agents' discovery among the steroid derivatives. *Steroids,* **144**: 52-65.
12. Sikharulidze M.I., Nadaraia N.Sh., Kakhabrishvili M.L., Barbakadze N.N., Mulkijanyan K.G. (2010) Biological activity of several steroidal oximes. *Chemistry of Natural Compounds,* **46:**493-494.
13. Sikharulidze M.I., Nadaraia N.Sh., Kakhabrishvili M.L., Labartkava M.O. (2007) Adamantane-containing 5α-steroids. *Chemistry of Natural Compounds,* **43:**753-755.
14. Nadaraia N.Sh., Sladkov V.I., Dorodnikova E.V., Mashkovskii M.D., Kemertelidze E.P. (1988) Synthesis and antiarrhythmic activity of epimeric 17-amino-5α-androstane-3-ols and their derivatives. *Khim.-Farm.Zh.,* **22**, 3:288-293.

Received June, 2021