ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis And Biological Activity Of Hydrazones Of 5α-Steroids.

Nanuli Sh Nadaraia[1], Nana N Barbakadze[1]*, Meri L Kakhabrishvili[1], and Vakhtang D Mshvildadze[1,2].

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

ABSTRACT

Condensation reactions catalyzed by acetic acid of severalarylhydrazine (2,4-dinitrophenyl hydrazine) and hydrazides (hydrazides of bromobenzoic and salicylic acid, benzofuran-2- and indol-2-carboxylicacid) with 3β-acetoxy- and 3β-hydroxy-5α-pregn-16-en-20-one (1 and 2), epiandrosterone (6) and 5α-androst-2-en-17-one (9) were studied for the purpose of synthesizing potentially bioactive 5α-steroidal hydrazones. The starting ketones (1,2,6,9) were synthesized on the bases of aglicon of steroidal saponine - tigogenin isolated from plant "Yucca gloriosa" introduced in Georgia. The structures of synthesized new hydrazones (3-5,7,8,10,11) were established by NMR ^{1}H, ^{13}C and mass-spectral data. The antiviral activity some of them (7,8,10,11) were studied.

Keywords: Steroid, hydrazone, synthesis, antiviral activity

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Steroidal compounds have attracted attention of pharmacologists, doctors, chemists and biochemists for many years. Due to some advantages of steroidal molecules their rational modification carried out which leads to change bioactivity to desirable direction. This is one of the best ways to create new medicines.

Among the synthetic biologically active steroidal compounds azasteroids – hydrazones, oximes and carbazones of pregnane, androstane, cholestane series have significant place, synthesis and biological activities (cytotoxic, antifungal, antibacterial, antiproliferative, antituberculosis, antiviral) of which derivatives are described in the literature [1-9]. Biological screening of this compounds showed, that insert of electron rich aromatic ring in the complex increases anticancer activities of them.

In order to detect of new biologically active steroidal compounds new hydrazones of the series of 5α-steroidal ketones were synthesized by us on the bases of tigogenin.

MATERIAL AND METHODS

^{1}H and ^{13}C NMR spectra were recorded from $CDCl_3$ and DMSO-d_6 solutions with TMS internal standard on a Bruker Avance 400 instrument (400.13 MHz for ^{1}H and 100.61 MHz for $_{13}C$). IR spectra were taken from KBr pellets on a Varian 660 FTIR spectrometer. Mass spectra were obtained on an HPLC-APCIMS (positive mode)-Agilent 1100 Series with an Inertsil PREP-ODS column (6.0 x 250 mm) and elution of steroids by H_2O–MeCN (20:80). Melting points were determined on a NAGEMA apparatus. The course of reactions and purity of synthesized compounds were monitored by TLC on Silufol UV-254 plates using C_6H_6–Me_2CO (10:1, 6:1). Chromatograms were detected using phosphomolybdic acid solution (10%) in EtOH followed by heating.

Chemical synthesis

General method for synthesis of compounds (3-5,7,8,10 and **11**)

A mixture of steroid (**1,2,6,9**) and the corresponding hydrazine (equal to the steroid weight) in ethanol was refluxed for 6–12 h with a catalytic amount of acetic acid. After completion of the reaction, the mixture was cooled to room temperature and poured into cold water. The solid separated was collected by filtration, washed with water, dried in air and crystallized from methanol.

2,4-Dinitrophenylhydrazone of 3β-acetoxy-5α-pregn-16-en-20-one**(3)**

Yield 78%; m.p. 232-234°C;^{1}H NMR (400.13 MHz, $CDCl_3$, δ, ppm J/Hz): 0.89(3H, s, 18-CH_3),1.05 (3H, s, 19-CH_3), 2.06(3H, s, 21-CH_3), 2.10(3H, s, $OCOCH_3$),4.65(1H, m, H-3), 6.29(1H, s, H-16), 7.85(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), 11.20(1H, s, NH).^{13}CNMR(100.61 MHz,$CDCl_3$,δ, ppm.): 12.2, 13.5, 16.3, 21.4, 21.5, 27.5, 28.5, 31.8, 32.0, 33.9, 34.0, 35.7, 36.0, 36.6, 44.9, 47.2, 54.6, 56.8, 73.7 (C-3), 116.6(C-6′), 123.6 (C-3′), 129.5 (C-2′), 130.2(C-5′), 136.7 (C-17), 137.8 (C-4′), 145.0(C-16), 151.0 (C-1′), 153.3 (C=N), 170.8(C=O). LC-MSm/z $[M+H]^+$ 539. $C_{29}H_{38}N_4O_6$.M.w. 538.

m-Bromobenzoilhydrazone of 3β-acetoxy-5α-pregn-16-en-20-one**(4)**

Yield 67%; m.p. 196-198°C;^{1}H NMR (400.13 MHz, $CDCl_3$, δ, ppm. J/Hz): 0.84(3H, s, 18-CH_3),0.99(3H, s, 19-CH_3), 1.59(3H, s, 21-CH_3), 2.02(3H, s, $OCOCH_3$),4.68(1H, m, H-3), 6.16(1H, s, H-16), 7.32(1H, m,H-Ar),7.65(2H, m,H-Ar), 7.98(1H, s, H-Ar),8.62(1H, s, NH-CO). ^{13}C NMR(100.61 MHz,$CDCl_3$,δ, ppm.):12.1, 15.9, 21.0, 21.2, 21.5, 27.5, 28.5, 31.8, 33.8, 34.0, 34.7, 35.3, 35.6, 36.5, 44.9, 46.9, 54.6, 56.9, 73.8 (C-3),121.5(C-3′), 128.4(C-6′), 129.3(C-2′), 130.5(C-5′), 133.1(C-4′), 133.9(C-1′), 135.2.(C-17), 146.8(C-16), 153.5(C=N), 169.1(NHCO), 170.8(C=O). LC-MSm/z $[M+H]^+$ 556. $C_{30}H_{39}BrN_2O_3$. M.w. 555.

Salicyloilhydrazone of 5α-pregn-16-en-3β-ol-20-one **(5)**

Yield 71%;m.p. 258-260°C;^{1}H NMR (400.13 MHz, DMSO-d_6, δ, ppm. J/Hz): 0.80 (3H, s, 18-CH_3),0.93(3H, s, 19-CH_3), 2.04(21-CH_3), 3.34(1H, m, H-3), 4.43(1H, s, 3-OH), 6.26(1H, s, H-16), 6.99(2H, m, H-Ar), 7.39(1H, t, J=6.9, H-Ar), 7.95(1H, d, J=7.5, H-Ar), 11.17 (1H, w.s, NH-CO).^{13}C NMR (100.61 MHz,DMSO-d_6, δ, ppm):12.5,

13.8, 16.3, 21.3,27.5,31.8, 31.9, 32.1, 33.9, 35.8, 36.9, 38.6,41.5,45.2, 47.2, 54.8, 57.0, 70.0(C-3), 117.4(C-1′), 118.5(C-3′), 119.8(C-5′), 130.9(C-6′), 133.6(C-4′), 134.5(C-17), 142.8(C-16),150.3(C=N), 154.1(C-2′), 162.3(NHCO). LC-MS m/z $[M+H]^+$ 451. $C_{28}H_{38}N_2O_3$. M.w. 450.

Benzofuran-2-ilhydrazone of 5α-androstan-3β-ol-17-one **(7)**

Yield 69%; m.p. 278-280°C. IR (KBr,ν,cm^{-1}): 3386-3198 (NH, OH),1662 (NHC=O), 1644 (C=N), 1594 (C=C Ar). ^{1}H NMR (400.13 MHz, $CDCl_3$,δ, ppm J/Hz): 0.85 (3H, s, 18-CH_3),0.97 (3H, s, 19-CH_3), 3.60 (1H, m, H-3), 7.31 (1H, t, J=7.6, H-Ar), 7.43 (1H, td, J =7.8, 1.2,H-Ar), 7.51 (1H, d, J =8.8, H-Ar), 7.61 (1H, s, H-Ar), 7.68 (1H, d, J =7.6, H-Ar), 8.94 (1H, v.s., NH-CO). LC-MSm/z $[M+H]^+$ 449.$C_{28}H_{36}N_2O_3$. M.w. 448.

Indol-2-ilhydrazone of 5α-androstan-3β-ol-17-one **(8)**

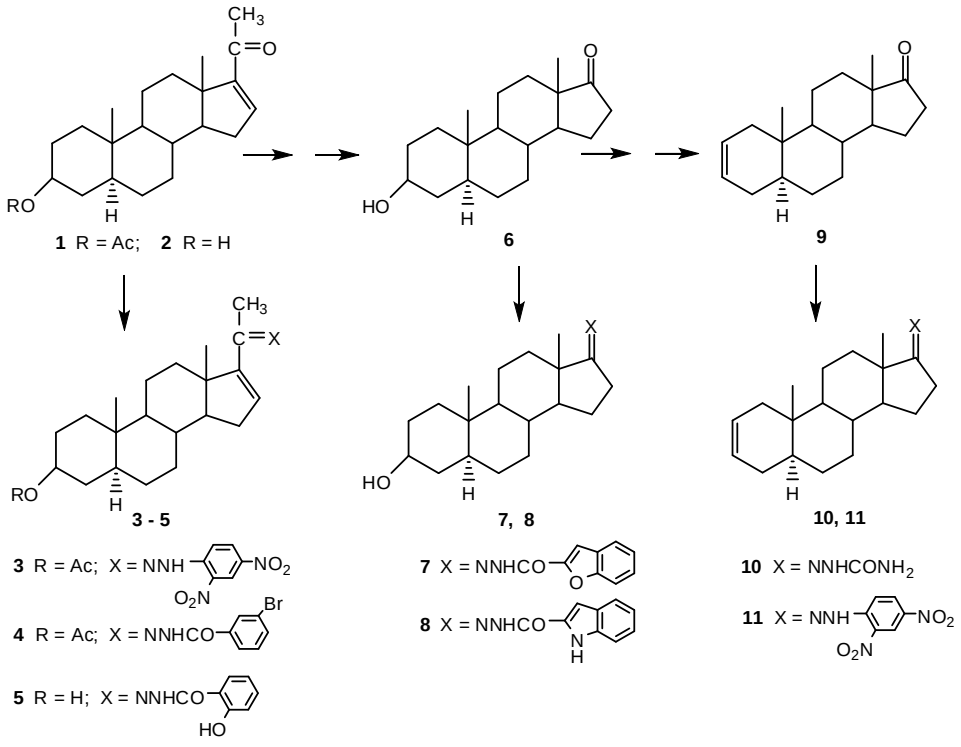
Yield 65%; m.p. 274-276 °C; IR (KBr,ν,cm^{-1}):3398-3183 (NH, OH),1669 (NHC=O), 1636 (C=N), 1582 (C=CAr). ^{1}H NMR (400.13 MHz, $CDCl_3$,δ, ppm J/Hz): 0.75 (3H, s, 18-CH_3),0.84 (3H, s, 19-CH_3), 3.61 (1H, m, H-3),7.14 (1H, t, J =7.2, H-Ar), 7.30 (1H, td, J =7.4, 1.2, H-Ar), 7.43 (1H, m, H-Ar), 7.58 (1H, s, H-Ar), 7.72 (1H, d, J =8.0, H-Ar),8.41 (1H, w.s, NH-CO), 10.18 (1H, w.s., NH). LC-MSm/z $[M+H]^+$ 448.$C_{28}H_{37}N_3O_2$. M.w. 447.

Semicarbazone of 5α-androst-2-en-17-one **(10)**

Yield 73%; m.p. 286-288 °C; IR (KBr,ν,cm^{-1}): 3462,3198(NH_2,NH), 1696(CO), 1640(C=N), 1572(C=C).^{1}H ЯМР (400.13 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.75 (3H, s, 18-CH_3),0.81 (3H, s, 19-CH_3), 2.15-2.36(2H, m, H-16), 5.59(2H, t, J=6.8, H-2, H-3), 5.99 (2H,w.s., NH_2), 8.61(1H, c, NH).LC-MSm/z $[M+H]^+$ 330. $C_{20}H_{31}N_3O$. M.w. 329.

2,4-Dinitrophenylhydrazone of 5α-androst-2-en-17-one **(11)**

Yield 70%; m.p. 238-240°C;IR (KBr,ν,$cm^{-1)}$: 3324(NH), 1616(C=N), 1560, 1517(Arom. ring, C=C), 1500 и 1332(Ar-NO_2). ^{1}H ЯМР(400.13 MHz, DMSO-d_6, δ, ppm. J/Hz): 0.80 (3H, s, 18-CH_3),0.97 (3H, s, 19-CH_3), 2.44-2.68(2H, m, H-16), 5.59(2H, t, J=6.8, H-2, H-3), 7.89(1H, d, J=9.5, H-Ar), 8.28(1H, dd, J=9.5, 2.5, H-Ar), 8.96(1H, d, J= 2.5, H-Ar), 10.66(1H, s, NH).LC-MSm/z $[M+H]^+$ 453.$C_{25}H_{32}N_4O_4$.M.w. 452.



Scheme 1: Synthesis of hydrazones of 5α-steroids 3-5, 7, 8, 10, 11

Antiviral screening

Antiviral activity of the synthesized compounds was studied in the framework of the international program "AACF Antiviral Testing in Animals" at the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, University of Utah, USA. In particular, all newly tested compounds were evaluated initially a) for inhibition of virus cytopathic effects (CPE assay) and b) for uptake of neutral red dye (NR Dye Uptake Assay) that stained undamaged virus cells. The color intensity was determined spectrophotometrically. The experimental results from a) were used to calculate the 50% inhibitory (cytotoxic) concentration CC_{50}; from b), the 50% effective concentration EC_{50}. Antiviral activity of each tested compounds was characterized by the selectivity index (SI_{50}), which was the ratio of CC_{50} to EC_{50}. As a rule, SI_{50} values of 10 or more indicated the presence of antiviral activity for the compound. The method was described in detail before[10].

RESULTS AND DISCUSSION

Chemistry

In continuation [11,12] of the search of biologically active 5α-steroids by interaction of 3β-acetoxy- and 3β-hydroxy-5α-pregn-16-en-20-one **(1,2)** with hydrochloride of 2,4-dinitrophenylhydrazine, hydrazides of bromobenzoic and salicylic acid corresponding hydrazones**(3-5)**were synthesized. After ketone **(1)** was transformed [13] into eriandrosterone**(6)**, from which by condensation with hydrazides of benzofuran-2- and indol-2-carboxylic acid hydrazones**(7,8)** were obtained.

According to the method [14] from 5α-androstan-3β-ol-17-one **(6)**5α-androst-2-en-17-one **(9)** was synthesized, by condensation of which with semicarbazide and 2,4-dinitrophenylhydrazine hydrochloride semicarbazone**(10)** and hydrazine **(11)** were obtained (Scheme 1).

The structures of the synthesized compounds **(3-5)** was established using ^{1}H-, ^{13}C-NMR and mass spectral data. In ^{1}H NMR spectrum of steroids **(3-5)** singlet signals of 18-CH_3, 19-CH_3 and 21-CH_3-groups were present at δ 0.89-0.80 ppm, 1.05-0.93 ppm and 2.06-1.59 ppm respectively. There were also signals of hydrogen atoms at C-16 with chemical shifts at 6.29-6.16 ppm. Aromatic protons were noted in the interval at 9.14-6.99 ppm, the signals of NH-groups - in the range of δ 11.20-8.62 ppm. The multiple 3α-protons of the 3β-ester groups of hydrazones**(3,4)** appeared at δ 4.65,4.68 ppm, 3β-hydroxy group of steroid **(5)**–at δ 3.34 ppm. In the ^{13}C NMR spectra of hydrazones(**3-5)** peaks of aromatic carbons existence in the interval 151.0-116.6, 133.9-121.5 and 154.1-117.4 ppm, signals C = N bonds –at 153.3,153.5 and 150.3 ppm. The C-16 peaks were observed at 145.0, 146.8 and 142.8 ppm, C-17 –136.7, 135.2 and 134.5 ppm, signals C-3 –at δ 73.7, 73.8 and 70.0 ppm respectively.

The structures of steroids **(7,8)** were proved using IR, ^{1}H NMR and mass spectral data. The infrared spectra of hydrazones**(7,8)** contained characteristic absorption bands of valence vibrations of the NH- and OH-groups in the range of 3386-3198 and 3398-3183 cm^{-1}, and the stretching vibrations of the carbonyl group for amides at 1662, 1669 cm^{-1}. The characteristic frequencies of the C=N bonds were observed at 1644, 1636, the aromatic C=C bonds — at 1594, 1582 cm^{-1} respectively. In the ^{1}H NMR spectra of compounds **(7,8)** signals of angular 18-CH_3 and 19-CH_3 groups were noted as singlets at δ 0.85, 0.75 and 0.97, 0.84 ppm. Aromatic protons were present in the range of 7.72-7.14 ppm, signals of protons of NH-groups appeared at 8.94, 10.18, multiple 3α-protons –at δ 3.60, 3.61 ppm respectively.

The structures of the derivatives **(10,11)** were confirmed by the data of IR, ^{1}H NMR and mass spectra. In the IR spectrum of semicarbazone**(10)** the absorption bands of the NH_2 and NH groups were observed at 3462, 3198 cm^{-1}, C=O groups at 1696 cm^{-1}, the absorption bands of stretching vibrations of the double C=N and C=C bonds at 1640 and 1572 cm^{-1} respectively. The IR spectrum of the hydrazine **(11)** contained absorption bands of valence vibrations of the NH- group at 3324 cm^{-1}, C=N bond – at 1616 cm^{-1}, aromatic and double C=C bonds — at 1560, 1517 cm^{-1}, and characteristic Ar–NO_2 stretching vibration bands at 1500 and 1332 cm^{-1}. In the ^{1}H NMR spectra (in DMSO-d6) of steroids **(10,11)** singlet signals of angular 18-CH_3 and 19-CH_3 groups were present at δ 0.75, 0.80 ppm and 0.81, 0.97 ppm. The signals of the protons at C-2 and C-3 of the double bond resonated as a distorted triplet at 5.59 ppm, and the singlet signals of the protons of NH-groups at δ 8.61 and 10.66 ppm respectively. The protons of the NH_2 group of the semicarbazone**(10)** manifested themselves in the

form of a broadened singlet at 5.99 ppm, the aromatic protons of the hydrazine **(11)**–in the region of 7.89-8.96 ppm. Molecular ions m/z $[M+H]^+$ of steroids **(3-5,7,8,10,11)** corresponded to the brutto formulas.

Antiviral evaluation

Antiviral activity of compounds **(7,8,10,11)** was studied in the Department of Microbiology and Infectious Diseases of the National Institute of Allergy and Infectious Diseases at the University of Utah, USA. The study showed that hydrazine **(7)** has a moderate and steroid **(8)** - weak antiviral activity against Poliovirus (cell culture Vero 76, strain Type 3, WM-3), the remaining steroids are inactive. For Rift Valley fever virus (Vero76 cell culture, strain MP-12) and for Influenza A virus H_1N_1 (MDCK cell culture, California strain 07.2009) only compound **(8)** has weak activity, the other steroids are inactive (Table 1).

Table 1: data for in vitro screening of antiviral activity of(7,8) and (10,11).

Compound	**Polio virus** (Pirodavir)			**Rift Valley fever virus** (Ribavirin)			**Influenza A virus H_1N_1**		
	EC_{50}	**EC_{50}**	**EC_{50}**	**EC_{50}**	**EC_{50}**	**EC_{50}**	**EC_{50}**	**EC_{50}**	**EC_{50}**
Visual (Cytopathic effect/toxicity)									
7	7.8	22	2.8	>68	68	0	34	>100	>2.9
8	0.56	3.2	5.7	3.7	28	7.6	5.6	37	6.6
10	2.8	3.2	1.1	3.2	8.6	2.7	>19	19	0
11	13	100	7.7	26	37	1.4	62	>100	>1.6
Control	0.32	>10	>31	6.8	>1000	>150	2.9	>100	>34
Neutral Red (Cytopathic effect/toxicity)									
7	4.5	>100	>22	>93	93	0	18	>100	>5.6
8	0.32	4.3	13	4	30	7.5	6.9	21	3
10	2.8	3.2	1.1	6.6	7.5	1.1	>12	>12	0
11	**8.7**	9.4	1.1	>14	14	0	72	>100	>1.4
Control	**0.3**	>10	>33	8.6	>1000	>120	3	>100	>33
EC_{50} is the compound concentration inhibiting virus replication by 50%; CC_{50}, compound concentration reducing cell survival by 50%; $SI_{50} = CC_{50}/EC_{50}$.									

CONCLUSION

Against Sarscorona virus, Takaribe virus, Respiratorysyncytial virus, Venezuelanequineencephalitis virus and Dengue virus (Cell cultures Vero 76, Vero, MA-104, Vero, Vero 76; strains (Urbani; TRVL-11573;A-2; TC-83; Type 2, New GuineaC) – all steroids **(7,8,10,11)**are inactive.

ACKNOWLEDGMENTS

This work was supported by Shota Rustaveli National Science Foundation (SRNSF) of Georgia (Grant №YS-2016-51, “Potential bioactive steroidal nitrogen-containing compounds”).

REFERENCES

[1] Gan C, Liu L, Cui J, Liu Z, Shi H, Lin Q, Sheng H, Yang C, Huang Y. Synthesis of Some Steroidal Derivatives with Side Chain of 20- and 22-Hydrazone Aromatic Heterocycles and their Antiproliferative Activity. Medicinal Chemistry, 2017; 13: 1-9.

[2] Mohareb RM, Al-Omran F, Reaction of pregnenolone with cyanoacetylhydrazine: Novel synthesis ofhydrazide–hydrazone, pyrazole, pyridine, thiazole, thiophene derivatives and their cytotoxicity evaluations.Steroids, 2012; **77**: 1551–1559.

[3] Loncle C, Brunel JM, Vidal N, Dherbomez M, Letourneux Y. Synthesis and antifungal activity of cholesterol-hydrazone derivatives. European Journal of Medicinal Chemistry, 2004; 39: 1067–1071.

[4] Khan SA, Kumar P, Joshi R, Iqbal PF, Saleem K, Synthesis and in vitro antibacterialactivity of new steroidal thiosemicarbazone derivatives. Eur.J.Med.Chem., 2008; 43(9): 2029-2034.
[5] Li J, Zhao X, Li L, Yuan Z, Tan F, Shi B, Zhang J. Design, synthesis and cytotoxic activity of a novel series of steroidalphenylpyrazoles.Steroids, 2016; 107:45–54.
[6] Cui J, Fan L, Huanga Y, Xin Y, Zhou A. Synthesis and evaluation of some steroidal oximes as cytotoxic agents:Structure/activity studies (II). Steroids, 2009; 74: 989–995.
[7] Gan C, Cui J, Su S, Lin Q, Jia L, Fan L, Huang Y. Synthesis and antiproliferative activity of some steroidalthiosemicarbazones, semicarbazones and hydrozones. Steroids, 2014; 87: 99–107.
[8] Merlani MI, Kemertelidze EP, Papadopoulos K, Men'shova NI. Some Derivatives of 5α-KetosteroidHydrazones: Synthesis from Tigogenin and Antituberculosis Activity. Russian J. Bioorganic Chemistry, 2004; 30 (5), p.497-501.
[9] Visbal G, San-Blas G, Maldonado A, Alvarez-Aular A, Capparelli MV, Murgich J. Synthesis, in vitro antifungal activity and mechanism of action of four sterol hydrazone analogues against the dimorphic fungus Paracoccidioides brasiliensis. Steroids. 2011; 76:1069– 1081.
[10] Assays for Antiviral Activity Against Respiratory and Biodefense Viruses. DMID, NIAID, NIH. http://arbidol.org/sidwell/ NIH_SOP_03-2006.
[11] Nadaraia NSh, Barbakadze NN, Kakhabrishvili ML, Silla B, Pichette A,Makhmudov US. Synthesis and Biological Activity of several Modified 5α-androstanolone Derivatives. Chemistry of Natural Compounds.2018; 54:310-314.
[12] Nadaraia NSh, Onashvili EO, Kakhabrishvili ML, Barbakadze NN, Sylla B, Pichette A. Synthesis and antiviral activity of several N-containing 5α-steroids. Chemistry of Natural Compounds. 2016; 52: 853-855.
[13] Men'shova NI, Korzinkina NA, Kemertelidze EP, Nadaraia NSh, Davitishvili MG, Lishcheta LI, GroshevaVS. Preparation of androstanolone acetate intermediate product synthesis of steroidal drugs from tigogenin (in Russian). Sb. Nauchn. Tr. VNIKhFI. 1982; 10: 83-85.
[14] Merlani MI, Davitishvili MG, Nadaraia NSh, Sikharulidze M.I, Papadopulos K. Conversion of epiandrosterone into 17β-amino-5α-androstane.Chemistry of Natural Compounds.2004; 40:144-146.