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Pharmacochemistry

Steroidal Hydrazones Containing an Adamantane Moiety

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(Presented by Academy Member Ether Kemertelidze)

Abstract. The conversion of the esterified steroidal ketone 3β-(1-adamantoate)-5α-androstan-17-one, derived from tigogenin, into a series of hydrazones was accomplished, and the cytotoxic, anti-inflammatory and antioxidant properties of the resulting compounds were investigated. Several of the synthesized hydrazones exhibited cytotoxic activity against lung carcinoma (A-549) cells, colorectal adenocarcinoma (DLD-1) cells, and normal skin fibroblasts (WS-1). Among them, 3β-(1-adamantanoate)-5α-androstan-17-one p-methylbenzoylhydrazone demonstrated the highest cytotoxic potency toward lung carcinoma cells. Evaluation of the anti-inflammatory activity of these steroids, based on inhibition of nitric oxide (NO) production in RAW 264.7 murine macrophages, revealed that 3β-(1-adamantoate)-5α-androstan-17-one p-chlorobenzoylhydrazone exhibited a pronounced anti-inflammatory effect. Antioxidant activity, determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, showed that among all synthesized compounds, only 3β-(1-adamantanoate)-5α-androstan-17-one salicyloylhydrazone displayed weak radical-scavenging activity. The structures of all synthesized hydrazones were confirmed by IR, ^{1}H NMR and mass spectral data. © *2025 Bull. Natl. Acad. Sci. Georg.*

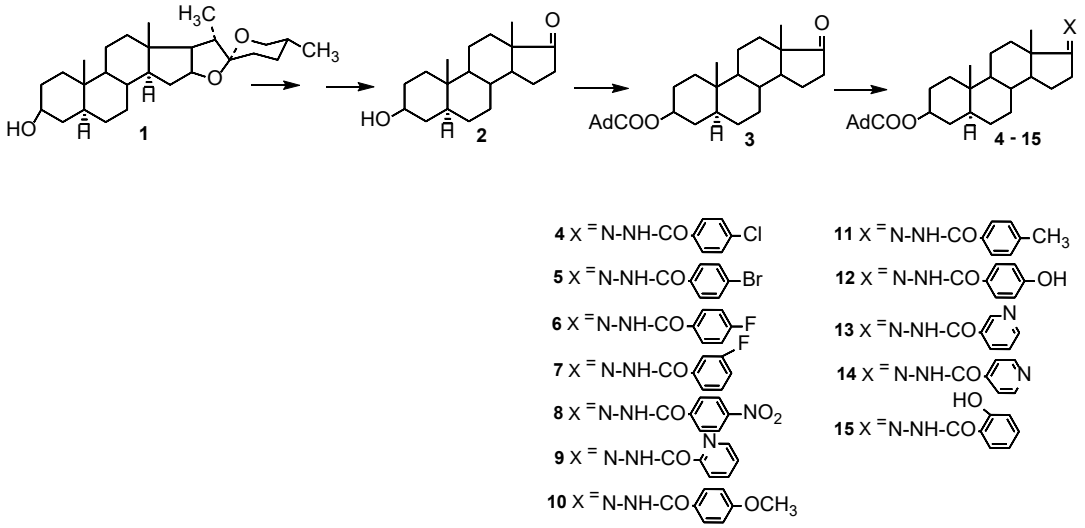
Keywords: steroid, hydrazone, ester, cytotoxic, anti-inflammatory, antioxidant activity

Introduction

The aim of this study was to synthesize and evaluate the biological properties of new steroidal hydrazones modified with an adamantane nucleus, as adamantane remains a key structural scaffold for the development of novel pharmaceutical agents (Sayadyan & Shklovsky, 2017). Moreover, the cytotoxic and antioxidant activities of hydrazones obtained via esterification of steroidal alcohols have been reported to be higher than those of their parent compounds. Given the pronounced biological activity of steroidal hydrazones, the design and synthesis of new derivatives based on this framework remain an important direction in the search for prototype therapeutic agents for the treatment of various diseases (Gan et al., 2014; Gaši et al., 2015).

Results and Discussion

Tigogenin (**1) – a** sapogenin isolated from the *Yucca gloriosa* plant introduced in Georgia, is a valuable raw material for the synthesis of steroid compounds (Kemertelidze & Pkheidze, 1972; Kavtaradze et al., 1988; Menshova et al., 1982). From its multi-step transformation product – epiandrosterone (**2),** the 3β-(1-adamantoate)-5α-androstan-17-one (**3)** was synthesized via esterification with 1-adamantanecarbonyl chloride. From ketone (**3),** several new hydrazones **(4-12)** and previously synthesized hydrazones (**13-15)** were obtained (Sikharulidze et al., 2007) (Scheme 1).



Scheme 1. Conversion of 3β-(1-adamantoate)-5α-androstan-17-one **3** to hydrazones **4-15** .

The structure of compounds **4-12** was determined by physicochemical (IR, ^{1}H NMR spectroscopy and mass spectrometry) methods.

The IR spectra of steroids **4–12** display absorption bands in the region of 3386–3178 cm^{-1}, characteristic of the NH stretching vibrations of the hydrazone group and at 2911-2904 and 2851-2847 cm^{-1} attributable to the CH bonds of the adamantane nucleus. Absorption bands corresponding to the stretching vibrations of the ester carbonyl (C=O) groups are observed at 1728–1720 cm^{-1}, while those of the amide carbonyl (NH–CO) groups appear at 1678–1659 cm^{-1}. The absorption bands of the C=N double bonds are found in the 1647-1642 cm^{-1} region, and those associated with the aromatic CH bonds are located at 1612-1585 cm^{-1}. For hydrazone **9**, the absorption bands characteristic of the pyridine ring appear at 1514 cm^{-1}. In the spectrum of compound **12**, additional bands corresponding to the stretching vibrations of OH- and NH-groups are observed at 3394 and 3256 cm^{-1}, respectively, while the hydrazone **8** exhibits two absorption bands of the Ar–NO_2 group at 1523 and 1342 cm^{-1}.

In the ^{1}H NMR spectra of hydrazones **4–12**, singlet signals of the 18-CH_3 and 19-CH_3 methyl groups appear in the δ 0.89–0.75 and 1.06–0.82 ppm regions, respectively. Singlets corresponding to protons of the adamantane fragment are found at δ 2.02–1.65 ppm. The multiplet signals of the 3α-protons of the 3β-ester groups are observed further downfield at δ 4.68–4.55 ppm, while the aromatic protons of the hydrazone fragment resonate in the δ 8.59–6.80 ppm region. The signals of the NHCO amide groups appear between δ 10.43–7.13 ppm. For compounds **10–12**, singlets corresponding to OCH_3-, CH_3-, and OH-groups are detected at δ 3.82, 2.36, and 9.89 ppm, respectively.

The molecular ion peaks m/z $[M+H]^+$ corresponded to the calculated molecular formulas of steroids **4-12**.

The cytotoxic activity of compounds **4–12** was evaluated against lung carcinoma (A-549), colorectal adenocarcinoma (DLD-1), and normal human skin fibroblast (WS-1) cell lines, with Daunorubicin as a reference drug. Hydrazones **7, 10–13** exhibited cytotoxic activity, with hydrazone **11** showing the highest potency against A-549 cells (IC_{50}=6.0±0.4 μM).

The anti-inflammatory potential of steroids **4–14** was assessed by measuring the inhibition of nitric oxide (NO) production in RAW 264.7 murine macrophages (ATCC TIB-71, Manassas, VA, USA). Among these, steroid **4** demonstrated a pronounced (40%) inhibitory effect, whereas compounds **5** and **14** exhibited moderate activities (27% and 23%, respectively). The IC_{50} values for compounds **4, 5**, and **14** were all above 80 μM.

The antioxidant activity of steroids **4–15** was determined using the DPPH (2,2-diphenyl-1-picryl-hydrazyl) spectrophotometric assay. Only hydrazone **15** displayed weak radical-scavenging activity.

Materials and Methods

^{1}H NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ on a Bruker Avance 400 MHz spectrometer using $SiMe_4$ as an internal standard. Mass spectra were obtained with an Agilent 1100 Series HPLC–APCI–MS (positive-ion mode) using an Inertsil Prep–ODS column (6.0 × 250 mm) and H_2O-CAN (20:80) as the mobile phase. Melting points were determined using a NAGEMA apparatus. The course of reactions and purity of the products were monitored by TLC on Silufol UV-254 plates, using benzene-acetone (4:1) and benzene-methanol (5:0.5) systems. Spots were visualized with a 10% phosphomolybdic acid solution in ethanol followed by heating.

Hydrazones (**4–15**) were synthesized according to the method described in reference (Sikharulidze et al., 2007).

3β-(1-adamantoate)-5α-androstan-17-one *p*-chlorobenzoylhydrazone (4). Yield 84%, mp 252-254°C. IR spectrum (KBr,ν,cm^{-1}): 3185(NH), 2905, 2850(CH-Ad), 1728(OCO), 1663(NHCO), 1647(C=N), 1593(aromatic nucleus). ^{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), 1.78(3H, s, H-Ad), 1.91(10H, s, H-Ad), 1.96(2H, s, H-Ad), 2.40(1H, m, H-16), 2.61(1H, m, H-16), 4.56(1H, m, H-3), 7.54(2H, d, J=8.1, H-Ar), 7.80(2H, d, J=8.2, H-Ar), 10.26(1H, s, NHCO). LC-MS m/z $[M+H]^+$ 605.5. $C_{37}H_{49}ClN_2O_3$. MM 604.5.

3β-(1-adamantoate)-5 α-androstan-17-one *p*-bromobenzoylhydrazone (5). Yield 86%, mp 247-249°C. IR spectrum (KBr,ν,cm^{-1}): 3178(NH), 2905, 2851(CH-Ad), 1728(OCO), 1665(NHCO), 1648(C=N), 1587(aromatic nucleus). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.87(3H, s, 18-CH_3), 1.06(3H, s, 19-CH_3), 1.79(3H, s, H-Ad), 1.92(10H, s, H-Ad), 1.97(2H, s, H-Ad), 2.33(1H, m, H-16), 2.59(1H, m, H-16), 4.55(1H, m, H-3), 7.68-7.82(4H, m, H-Ar), 10.27(1H, s, NHCO). LC-MS m/z $[M+H]^+$ 650. $C_{37}H_{49}BrN_2O_3$. MM 649.

3β-(1-adamantoate)-5 α-androstan-17-one *p*-fluorobenzoylhydrazone (6). Yield 92%, mp 238-240°C. IR spectrum (KBr,ν,cm^{-1}): 3197(NH), 2904, 2852(CH-Ad), 1721(OCO), 1659(NHCO), 1646(C=N), 1602(aromatic nucleus). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.89(3H, s, 18-CH_3), 0.98(3H, s, 19-CH_3), 1.73(4H, s, H-Ad), 1.89(7H, s, H-Ad), 2.02(4H, s, H-Ad), 2.31(1H, m, H-16), 2.47(1H, m, H-16), 4.68(1H, m, H-3), 7.13(1H, s, NHCO), 7.81-8.31(4H, m, H-Ar). LC-MS m/z $[M+H]^+$ 589. $C_{37}H_{49}FN_2O_3$. MM 588.

3β-(1-adamantoate)-5α-androstan-17-one *m*-fluorobenzoylhydrazone (7). Yield 70%, mp 243-245°C. IR spectrum (KBr,ν,cm^{-1}): 3245(NH), 2911, 2847(CH-Ad), 1725(OCO), 1659(NHCO), 1646(C=N), 1585(aromatic nucleus). 1H NMR spectrum (400 MHz, DMSO-d_6 , δ, ppm, J/Hz): 0.83(3H, s, 18-CH_3), 0.87(3H, s, 19-CH_3), 1.67(6H, s, H-Ad), 1.79(6H, s, H-Ad), 1.96(3H, s, H-Ad), 2.41(1H, m, H-16), 2.60(1H, m, H-16), 4.56(1H, m, H-3), 7.37-7.65(4H, m, H-Ar), 10.28(1H, s, NHCO). LC-MS m/z [M+H] $^+$ 589. $C_{37}H_{49}FN_2O_3$. MM 588.

3β-(1-adamantoate)-5α-androstan-17-one *p*-nitrobenzoylhydrazone (8). Yield 60%, mp 268-270°C. IR spectrum (KBr,ν,cm^{-1}): 3386(NH), 2909, 2850(CH-Ad), 1720(OCO), 1662(NHCO), 1642(C=N), 1601(aromatic nucleus), 1523 and 1342(Ar-NO2). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.88(3H, c, 18-CH_3), 0.99(3H, s, 19-CH_3), 1.73(7H, s, H-Ad), 1.88(5H, s, H-Ad), 2.02(3H, s, H-Ad), 2.32(1H, m, H-16), 2.49(1H, m, H-16), 4.67(1H, m, H-3), 7.98-8.32(4H, m, H-Ar), 8.45(1H, s, NHCO). LC-MS m/z [M+H]$^+$ 616. $C_{37}H_{49}N_3O_5$. MM 615.

3β-(1-adamantoate)-5α-androstan-17-one 2-pyridinecarbohydrazone (9). Yield 62%, mp 281-283°C. IR spectrum (KBr,ν,cm^{-1}): 3329(NH), 2908, 2851(CH-Ad), 1723(OCO), 1696(NHCO), 1645(C=N) , 1514 (Pyridine ring). ^{1}H NMR spectrum (DMSO-d_6 , δ, ppm, J/Hz): 0.88(3H, c, 18-CH_3), 0.97(3H, s, 19-CH_3), 1.67(3H, s, H-Ad), 1.79(10H, s, H-Ad), 1.96(2H, s, H-Ad), 2.39(1H, m, H-16), 2.61(1H, m, H-16), 4.56(1H, m, H-3), 7.50(1H, m, H-Ar), 7.92(1H, t, J=8.4, H-Ar), 8.32(1H, d, J=7.7, H-Ar), 8.59(1H, d, J=4.3, H-Ar) 10.43(1H, s, NHCO). LC-MS m/z [M+H]$^+$ 572. $C_{36}H_{49}N_3O_3$. MM 571.

3β-(1-adamantoate)-5 α-androstan-17-one *p*-methoxybenzoylhydrazone (10). Yield 82%, mp 251-253°C. IR spectrum (KBr, ν ,cm $^{-1}$): 3268(NH), 2904, 2852(CH-Ad), 1722(OCO), 1678(NHCO), 1646(C=N), 1606(aromatic nucleus). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm , J/Hz): 0.83(3H, c, 18-CH_3), 0.86(3H, s, 19-CH_3), 1.67(6H, s, H-Ad), 1.79(6H, s, H-Ad), 1.96(3H, s, H-Ad), 2.39(1H, m, H-16), 2.62(1H, m, H-16), 3.82(3H, s, OCH_3), 4.56(1H, m, H-3), 7.0(2H, d, J=8.2, H-Ar), 7.80(2H, d, J=8.3, H-Ar), 10.02(1H, s, NHCO). LC-MS m/z [M+H] $^+$ 601. $C_{38}H_{52}N_2O_4$. MM 600.

3β-(1-adamantoate)-5α-androstan-17-one *p*-methylbenzoylhydrazone (11). Yield 68%, mp 218-220°C. IR spectrum (KBr,ν,cm^{-1}): 3259(NH), 2907, 2851(CH-Ad), 1723(OCO), 1659(NHCO), 1643(C=N), 1612(aromatic nucleus). ^{1}H NMR spectrum (400 MHz, DMSO-d_6 , δ, ppm , J/Hz): 0.83(3H, c, 18-CH_3), 0.86(3H, s, 19-CH_3), 1.67(5H, s, H-Ad), 1.79(7H, s, H-Ad), 1.96(3H, s, H-Ad), 2.36 (3H, s, CH_3-Ar), 2.40(1H, m, H-16), 2.60(1H, m, H-16), 4.56(1H, m, H-3), 7.27(2H, d, J=8.2,H-Ar), 7.70(2H, d, J=8.3,H-Ar), 10.08(1H, s, NHCO). LC-MS m/z [M+H] $^+$ 585. $C_{38}H_{52}N_2O_3$. MM 584.13

3β-(1-adamantoate)-5α-androstan-17-one *p*-hydroxybenzoylhydrazone (12). Yield 65%, mp 291-293°C. IR spectrum (KBr,ν,cm^{-1}): 3394, 3256(OH, NH), 2911, 2852(CH-Ad), 1721(OCO), 1659(NHCO), 1639(C=N), 1608(aromatic nucleus). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm , J/Hz): 0.83(3H, c, 18-CH_3), 0.85(3H, s, 19-CH_3), 1.67(6H, s, H-Ad), 1.79(6H, s, H-Ad), 1.96(3H, s, H-Ad), 2.38(1H, m, H-16), 2.59(1H, m, H-16), 4.56(1H, m, H-3), 6.80(2H, d, J=8.2,H-Ar), 7.68(2H, d, J=8.3,H-Ar), 9.89(1H, s, OH-C_6H_4), 10.05(1H, s, NHCO). LC-MS m/z [M+H] $^+$ 587. $C_{37}H_{50}N_2O_4$. MM 586.

Conclusion

Structure–activity relationship analysis indicated that the presence of a p-mehylbenzoylhydrazone fragment at position **17** of the steroid nucleus (compound **11**) was most favorable for cytotoxic activity among the esterified hydrazones **4-14**, whereas the p-chlorobenzoylhydrazone moiety (steroid **4**) contributed most to the anti-inflammatory properties.

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ფარმაკოქიმია

ადამანტანის ფრაგმენტის შემცველი სტეროიდული ჰიდრაზონები

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(წარმოდგენილია აკადემიის წევრის ე. ქემერტელიძის მიერ)

გამოკვლეულია ტიგოგენინიდან მიღებული ესტერიფიცირებული სტეროიდული კეტონის, 3β-(1-ადამანტოატ)-5α-ანდროსტან-17-ონის ჰიდრაზონებად გარდაქმნის შესაძლებლობა და მიღებული ნაერთების ციტოტოქსიკური, ანთების საწინააღმდეგო და ანტიოქსიდანტური აქტიურობა. რამდენიმე მათგანი ხასიათდება ზოგადი ციტოტოქსიკური აქტიურობით ფილტვის კარცინომის A-549, კოლორექტალური ადენოკარცინომის DLD-1 და კანის ნორმალური ფიბრობლასტების WS-1 მიმართ. მათ შორის, ყველაზე მაღალი ციტოტოქსიკური აქტიურობა ფილტვის კარცინომის მიმართ ახასიათებს 3β-(1-ადამანტოატ)-5α-ანდროსტან-17-ონის პ-მეთილბენზოილჰიდრაზონს. სტეროიდების ანთების საწინააღმდეგო აქტიურობის შეფასებამ NO-ს წარმოების ინჰიბირებით RAW 264.7 თაგვის მაკროფაგებზე აჩვენა 3β-(1-ადამანტოატ)-5α-ანდროსტან-17-ონის პ-ქლორბენზოილჰიდრაზონის გამოხატული ანთების საწინააღმდეგო ეფექტურობა. DPPH (2,2-დიფენილ-1-პიკრილჰიდრაზილი) მეთოდით ანტიოქსიდანტური აქტიურობის განსაზღვრით ყველა სინთეზირებული ნაერთიდან რადიკალების შთანთქმის სუსტი აქტიურობა აღმოაჩნდა მხოლოდ 3β-(1-ადამანტოატ)-5α-ანდროსტან-17-ონის სალიცილოილჰიდრაზონს. სინთეზირებული ჰიდრაზონების აღნაგობა დადგენილია იწ-, 1H ბმრ- და მას-სპექტრების მონაცემების საფუძველზე.

REFERENCES

Gan, Ch., Cui, J., Su, Sh., Lin, Q., Jia, L., Fan, L., & Huang, Y. (2014). Synthesis and antiproliferative activity of some steroidal thiosemicarbazones, semicarbazones and hydrazones. *Steroids, 87,* 99-107. doi:10.1016/j.steroids.2014.05.026.

Gaši, K.M.P., Djurendic, E.A., Szecsi, M., Gardi, J., Csanadi, J.J., Klisuric, O.R., Dojcinovic-Vujaškovic, S.V., Nikolic, A.R., Savic, M.P., Ajdukovic, J.J., Oklješa, A.M., Kojic, V.V., Sakac, M.N., & Jovanovic-Šanta, S.S. (2015). Microwave assisted synthesis and biomedical potency of salicyloyloxy and 2-methoxybenzoyloxy androstane and stigmastane derivatives. *Steroids, 94,* 31-40. https://doi.org/10.1016/j.steroids.2014.12.008

Kavtaradze, L.K., Dabrundashvili, R.I., Menshova, N.I., Korzinkina, N.A., & Kemertelidze, E.P. (1988). Synthesis of 3β-acetoxy-5α-pregn-16-en-20-one from tigogenin. *Report of the Academy of Sciences of Georgia*, *132*, 537-539.

Kemertelidze, E.P., & Pkheidze, T.A. (1972). Tigogenin from *Yucca gloriosa* a possible raw-material for synthesis of steroid hormonal preparations. *Chemical-Pharmaceutical Journal, 6*(12), 44-47.

Menshova, N.I., Korzinkina, N.A., Kemertelidze, E.P., Nadaraia, N.Sh., Davitishvili, M.G., Lisheta, L.I., & Grosheva, V.S. (1982). Obtaining androstanolone acetate – a semi-product of steroid drug synthesis from tigogenin. *Collection of Scientific Papers*, *10,* 83-86. All-Union Scientific Research Chemical-Pharmaceutical Institute.

Sayadyan, Kh.S., & Shklovsky, V.M. (2017). Adamantane-based medications. *Neurology Psychiatry*, *1*(130), 59-63.

Sikharulidze, M.I., Nadaraia, N.Sh., Kakhabrishvili, M.L., & Labartkava, M.O. (2007). Adamantane-containing 5α-steroids. *Chemistry of Natural Compounds, 43*(6), 753-755. Doi:10.1007/s10600-007-0258-z.

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