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SYNTHESIS AND ANTIVIRAL ACTIVITY OF MODIFIED 5α-STEROIDS

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Hydrazones are a class of organic compounds possessing various biological activities [1, 2].

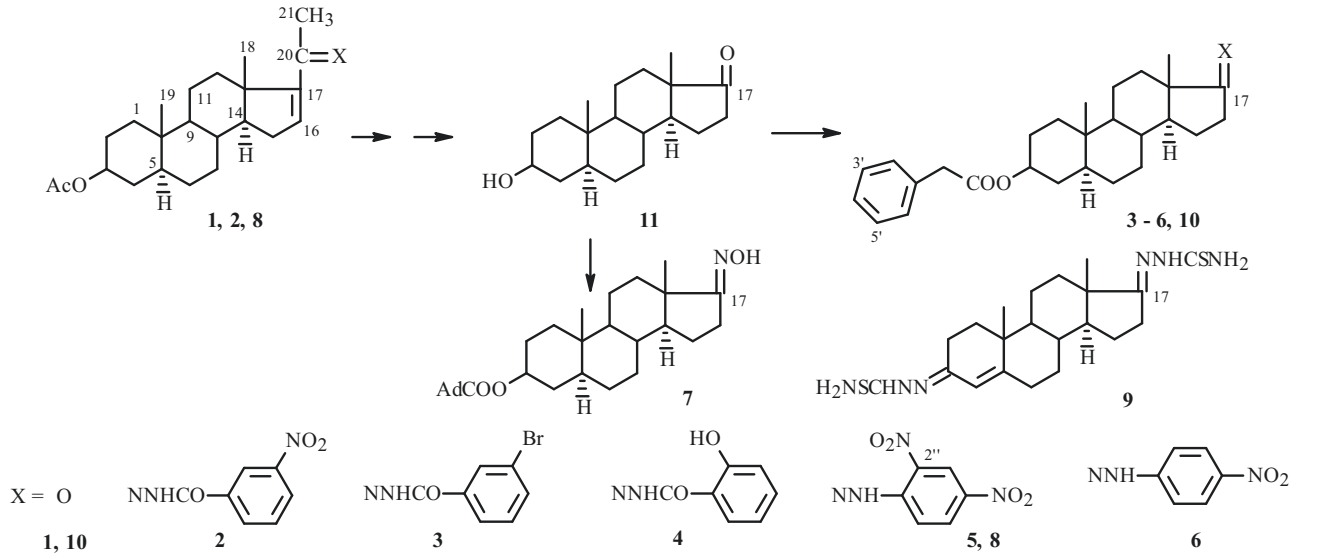
Steroid oximes, thiosemicarbazones, semicarbazones, and hydrazones have attracted interest because of their high pharmacological activity, e.g., antibacterial, antiviral, antitumor, etc. [3–6].

Transformation into ester derivatives is a common method of structural modification of steroids. Addition of an ester into the steroid core affects the biological activity. The cytotoxicity of several steroid esters synthesized via esterification of a hydroxyl group was shown to be significantly less than that of their precursors [7].

Previously, we reported that several epiandrosterone hydrazones modified by phenylacetic acid chloride exhibited antiviral activity [8].

In continuation of research on the synthesis of new biologically active steroids, several new (**2**–**7**) and previously known hydrazones (**8** and **9**) [9, 10] were prepared from 5α-pregnenolone acetate **1** and tested for antiviral activity. Compound **2** was synthesized from steroid **1**; hydrazones **3**–**6**, from ketone **10** via reaction with hydrazides or hydrazines (hydrazides of *m*-nitrobenzoic, *m*-bromobenzoic, and salicylic acids and 2,4-dinitrophenyl- and *p*-nitrophenylhydrazine) in EtOH in the presence of a catalytic amount of AcOH. Oxime **7** was obtained from 3β-(1-adamantoate)-5α-androstan-17-one; this ketone and starting **10**, via esterification of epiandrosterone **11** by the literature method [8].

The structures of the synthesized steroids **2**–**7** were confirmed using IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. IR spectra of **2**, **3**, **6**, and **7** contained absorption bands for ester C=O in the range 1750–1668 cm^{-1}; for NHCO (hydrazones **2** and **3**), at 1667 and 1665 cm^{-1}; for stretching vibrations of C=N and aromatic C=C double bonds (steroids **2**, **3**, and **6**), at 1640–1602 and 1562–1560 cm^{-1}, respectively; for hydrazone NH of **6** and OH and C=N of oxime **7**, at 3373, 3278, and 1658 cm^{-1}, respectively. IR spectra of nitro derivatives **2** and **6** also had characteristic Ar–NO_2 stretching vibrations at 1500 and 1506 and 1374 and 1375 cm^{-1}, respectively.



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The ^{1}H NMR spectra of **2**–**7** exhibited singlets for CH_3-18 and CH_3-19 methyls at δ 0.95–0.85 and 1.05–0.89 ppm, respectively. The aromatic protons of **2**–**6** had chemical shifts (CSs) in the range 9.11–6.85 ppm. Singlets for the methylene protons of the phenylacetoxy groups of hydrazones **3**–**6** were observed at δ 3.51; the resonances of NH groups of steroids **5** and **6**, at 10.76 and 9.30; the NHCO protons of **2**–**4**, in the range δ 9.00–8.35 ppm. Multiplets of the 3α protons from the 3β esters of steroids **2**–**7** showed CSs at δ 4.65–4.59 ppm. A broad singlet from the C=N–OH proton of oxime **7** appeared at δ 7.43 ppm. Resonances of other protons agreed with the proposed structures. ^{13}C NMR spectra of hydrazones **4** and **5** had C-3 resonances at δ 74.0 ppm; aromatic C atoms, in the range δ 154.9–116.4; of C=N bonds, δ 171.2–161.9; of O–C=O groups, at δ 171.2 and 172.3 ppm, respectively. The resonance of the NH–CO C atom of **4** had a CS of δ 158.8 ppm.

Mass spectra confirmed the empirical formulas of **2**–**7**.

Screening for specific antiviral activity of **6** was performed by the National Institute of Allergy and Infectious Diseases at the University of Utah (USA) using the following virus strains: *Polio virus* (Vero 76 cell culture, strain Type 3, WM-3); *SARS-corona virus* (Vero 76 cell culture, strain Urbani); *Rift Valley fever virus* (Vero 76 cell culture, strain MP-12); *Tacaribe virus* (Vero cell culture, strain TRVL-11573); *Venezuelan equine encephalitis virus*, *Respiratory syncytial virus*, *Influenza A virus* H_1N_1, *Dengue virus* (Vero cell culture, MA-104, MDCK; Vero 76; strains TC-83, A-2, California 07/2009, Type 2, New Guinea C, respectively); and *Cytomegalovirus* (strains Davis and AD-169) and *varicella-zoster virus* (strains OKA and 07-1); of compounds **2**–**5** and **7**–**9**, at Rega Institute for Medical Research, Belgium. As it turned out, only hydrazone **6** exhibited moderate antiviral activity against *Polio virus*. All other compounds did not possess any significant activity against these strains.

Steroids **2**–**6** and **8** were prepared by the literature method [11]; oxime **7**, as before [8]; androst-4-en-3,17-dione *bis*-thiosemicarbazone **9**, by the literature method [10].

3β-Acetoxy-5α-pregn-16-en-20-one *m*-Nitrobenzoylhydrazone (2). Yield 72%, mp 186–188°C. IR (KBr, ν, cm^{-1}): 3425 (NH), 1750 (C=O), 1667 (NH-CO), 1640 (C=N), 1561 (arom. ring), 1500 and 1374 (Ar-NO_2). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.89 (3H, s, CH_3-18), 1.05 (3H, s, CH_3-19), 1.96 (3H, s, CH_3-21), 2.07 (3H, s, $OCOCH_3$), 4.59 (1H, m, H-3), 6.19 (1H, s, H-16), 7.61 (1H, t, J = 8.2, H-5′), 8.10 (1H, d, J = 7.1, H-6′), 8.35 (1H, d, J = 7.3, H-4′), 8.64 (1H, s, H-2′), 9.00 (1H, br.s, NHCO). LC-MS, *m/z* 522 [M + H]$^+$. $C_{30}H_{39}N_3O_5$. MW 521.

3β-Phenylacetoxy-5α-androstan-17-one *m*-Bromobenzoylhydrazone (3). Yield 81%, mp 159–161°C. IR (KBr, ν, cm^{-1}): 3412 (NH), 1729 (C=O), 1665 (NH-CO), 1602 (C=N), 1562 (arom. ring). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.85 (3H, s, CH_3-18), 0.95 (3H, s, CH_3-19), 2.18–2.25 (2H, m, H-16), 3.51 (2H, s, $\underline{CH_2}C_6H_5$), 4.61 (1H, m, H-3), 7.24–7.34 (5H, m, C_6H_5), 7.52–8.25 (4H, ArH), 8.35 (1H, br.s, NHCO). LC-MS, *m/z* 606 [M + H]$^+$. $C_{34}H_{41}BrN_2O_3$. MW 605.

3β-Phenylacetoxy-5α-androstan-17-one Salicyloylhydrazone (4). Yield 67%, mp 254–256°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.95 (3H, s, CH_3-18), 0.98 (3H, s, CH_3-19), 2.40–2.51 (2H, m, H-16), 3.51 (2H, s, $\underline{CH_2}C_6H_5$), 4.61 (1H, m, H-3), 6.85–7.41 (9H, ArH), 8.65 (1H, br.s, NH-CO). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.2, 16.9, 20.7, 23.4, 25.2, 27.4, 28.3, 31.4, 33.8, 33.9, 34.9, 35.7, 36.7, 41.8, 44.6, 45.3, 53.3, 54.4, 74.0 (C-3), 117.2 (C-1″), 117.6 (C-3″), 118.6 (C-5″), 125.0 (C-6″), 126.9 (C-4′), 128.5 (C-3′, 5′), 129.2 (C-2′, 6′), 134.3 (C-4″), 134.5 (C-1′), 154.9 (C-2″), 158.8 (NHCO), 161.9 (C=N), 171.2 (O–C=O). LC-MS, *m/z* 543 [M + H]$^+$. $C_{34}H_{42}N_2O_4$. MW 542.

3β-Phenylacetoxy-5α-androstan-17-one 2,4-Dinitrophenylhydrazone (5). Yield 76%, mp 198–200°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.87 (3H, s, CH_3-18), 0.95 (3H, s, CH_3-19), 2.50–2.65 (2H, m, H-16), 3.51 (2H, s, $\underline{CH_2}C_6H_5$), 4.61 (1H, m, H-3), 7.26–7.34 (5H, m, C_6H_5), 7.93 (1H, d, J = 9.5, ArH), 8.28 (1H, dd, J = 9.5, 2.5, ArH), 9.11 (1H, d, J = 2.5, ArH), 10.76 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 12.3, 17.2, 20.7, 23.5, 26.4, 27.4, 28.3, 31.4, 33.9, 34.1, 35.0, 35.7, 36.7, 41.8, 44.7, 45.3, 53.5, 54.4, 74.0 (C-3), 116.4 (C-6″), 123.6 (C-3″), 126.9 (C-4′), 128.5 (C-3′, 5′), 128.8 (C-2″), 129.2 (C-2′, 6′), 129.9 (C-5″), 134.3 (C-1′), 137.5 (C-4″), 145.4 (C-1″), 171.2 (C=N), 172.3 (O-C=O). LC-MS, *m/z* 589 [M + H]$^+$. $C_{33}H_{40}N_4O_6$. MW 588.

3β-Phenylacetoxy-5α-androstan-17-one *p*-Nitrophenylhydrazone (6). Yield 83%, mp 221–223°C. IR (KBr, ν, cm^{-1}): 3373 (NH), 1668 (C=O), 1615 (C=N), 1560 (arom. ring), 1506 and 1375 (Ar-NO_2). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.85 (3H, s, CH_3-18), 0.89 (3H, s, CH_3-19), 2.38–2.50 (2H, m, H-16), 3.51 (2H, s, $\underline{CH_2}C_6H_5$), 4.61 (1H, m, H-3), 7.10 (2H, d, J = 9.0, ArH), 7.18–7.28 (5H, m, C_6H_5), 8.00 (2H, d, J = 9.1, ArH), 9.30 (1H, s, NH), LC-MS, *m/z* 544 [M + H]$^+$. $C_{33}H_{41}N_3O_4$. MW 543.

17-Hydroximino-3β-(1-adamantoate)-5α-androstane (7). Yield 87%, mp 245–247°C. IR (KBr, ν, cm^{-1}): 3278 (OH), 2924, 2851 (CH-Ad), 1731 (C=O), 1658 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.87 (3H, s, CH_3-18), 0.89 (3H, s, CH_3-19), 1.74 (3H, s, CH-Ad), 1.88 (10H, s, CH_2-Ad), 1.99 (2H, s, CH_2-Ad), 2.40–2.55 (2H, m, H-16), 4.65 (1H, m, H-3), 7.43 (1H, br.s, C=N-OH). LC-MS, *m/z* 468 [M + H]$^+$. $C_{30}H_{45}NO_3$. MW 467.

REFERENCES

1. S. Chen, J. Cui, Y. Li, and L. Fan, *Chin. J. Org. Chem.*, **31** (2), 187 (2011).
2. G. Verma, A. Marella, M. Shaquiquzzaman, M. Akhtar, M. R. Ali, and M. M. Alam, *J. Pharm. Bioallied Sci.,* **6** (2), 69 (2014).
3. Ch. Gan, J. Cui, Sh. Su, Q. Lin, L. Jia, L. Fan, and Y. Huang, *Steroids*, **87**, 99 (2014).
4. S. A. Khan, P. Kumar, R. Joshi, P. F. Iqbal, and K. Saleem, *Eur. J. Med. Chem*., **43**, 2029 (2008).
5. M. Alam and D. U. Lee, *Korean J. Chem. Eng*., **32** (6), 1142 (2015).
6. Ch. Gan, L. Liu, J. Cui, Zh. Liu, H. Shi, Q. Lin, H. Sheng, Ch. Yang, and Y. Huang, *Med. Chem.*, **13**, 375 (2017).
7. Y. Huang, H. Wen, J. Zheng, Ch. Gan, L. Pang, Ch. Pang, X. Liu, J. Zhan, and J. Cui, *Nat. Prod. Res.*, 1 (2018).
8. N. Sh. Nadaraia, N. N. Barbakadze, M. L. Kakhabrishvili, B. Sylla, A. Pichette, and U. S. Makhmudov, *Chem. Nat. Compd.,* **54**, 310 (2018).
9. N. Sh. Nadaraia, N. N. Barbakadze, M. L. Kakhabrishvili, and V. D. Mshvildadze, *Res. J. Pharm., Biol. Chem. Sci.,* **10** (1), 239 (2019).
10. M. B. Zivkovic, I. Z. Matic, M. V. Rodic, I. T. Novakovic, D. M. Sladic, and N. M. Krstic, *RSC Adv.,* **6** (41), 34312 (2016).
11. N. Sh. Nadaraia, M. L. Kakhabrishvili, N. N. Barbakadze, V. D. Mshvildadze, K. G. Mulkidzhanyan, and A. Pichette, *Chem. Nat. Compd.*, **57**, 395 (2021).