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SYNTHESIS AND ANTITUMOR ACTIVITY OF SOME 5α-STEROID DERIVATIVES

M. I. Merlani,[1*] L. Sh. Amiranashvili,[1]
K. G. Mulkidzhanyan,[1] and A. R. Shelar[2]

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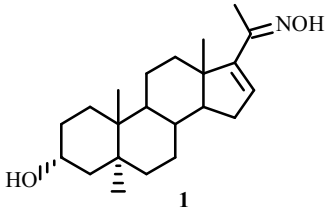
Recently we reported the synhesis and biological activity of some 5α-androstane derivatives that exhibit pronounced antitumor activity toward certain cancer cell lines [1]. In continuation of the search for highly effective compounds in this series, we synthesized new 5α-androstane and 5α-pregnane derivatives and studied their antitumor activity. Oximes (**1**-**4**) were prepared from the corresponding ketosteroids 3α-hydroxy-5α-pregn-16-en-20-one (**5**), 3α-hydroxy-5α-pregnan-20-one (**6**), 3α-hydroxy-5α-androst-9(11)-en-17-one (**7**), and androst-1,4-dien-3,17-dione (**8**) by reacting them with hydroxylamine in pyridine. The starting ketones (**5**-**8**) were synthesized beforehand by the literature method [2]. Aminonitrile **9** was synthesized as previously described [3].



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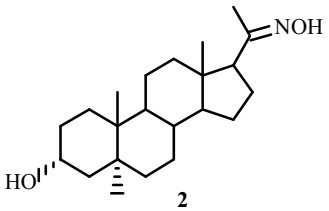

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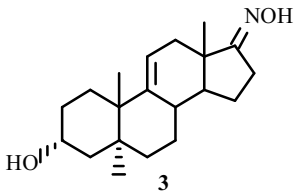

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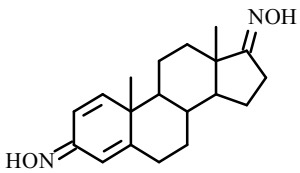

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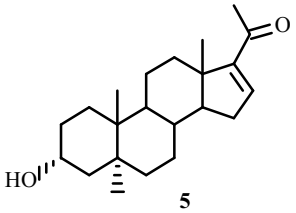

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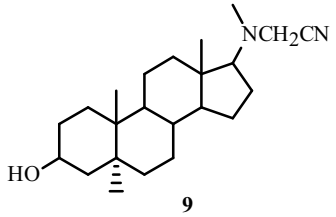

9

Structures of the known and previously unknown compounds were confirmed by PMR and IR spectroscopy and elemental analysis.

Antitumor activity was studied in HeLa cell culture. Compounds **1**-**5** and **9** were investigated. Ketosteroid **5** and aminonitrile **9** showed high activity. Oximes **2** and **4** were relatively less active. Oximes **1** and **3** were inactive even at doses of 100 μg/mL. These biological tests showed that both aminonitrile **9** and ketosteroid **5** are interesting for further expanded testing.

3α-Hydroxy-20-hydroximino-5α-pregn-16-ene (1). Compound **5** (2 g, 6.32 mmol) and $NH_2OH·HCl$ (0.43 g, 6.32 mmol) in pyridine (10 mL) were heated at 65-67°C for 3 h, cooled to 20°C, and poured into icewater (100 mL). The precipitate was filtered off, washed with water, and dried to afford **1** (2.98 g, 90%), mp 182-183°C (MeOH). IR spectrum (KBr, ν_{max}, cm^{-1}): 3286 (OH), 1690 (C=N), 1640 (C=C). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm): 0.68 (3H, s, CH_3-10), 0.79 (3H, s, CH_3-13), 1.79 (3H, s, CH_3-20), 3.78 (1H, br.s, OH), 3.91 (1H, s, Hβ-3), 10.35 (br.s, CN–OH). ^{13}C NMR spectrum (δ, ppm): 11.8 (C-18), 16.6 (C-19), 21.1 (C-21), 65.2 (C–OH), 131.4 (C-16), 158.2, 152.8 (C-17), 180.5 (C=N).

3α-Hydroxy-17-hydroximino-5α-androst-9(11)-ene (3). Oxime **3** was prepared analogously to **1** from **7** (1 g, 3.46 mmol) and $NH_2OH·HCl$ (0.24 g, 3.46 mmol) in pyridine (7 mL). Yield of **3**, 0.96 g (92%), mp 238-240°C (MeOH).

1) I. G. Kutateladze Institute of Pharmaceutical Chemistry, Academy of Sciences of Georgia, Tbilisi, 0159, ul. Saradzhishvili, 36, fax: (99532) 52 00 23, e-mail: maiamer@mail.ru; 2) Pharmaceutical College, University KLES, Belgaum-10, India. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 659-660, November-December, 2008. Original article submitted July 10, 2008.

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IR spectrum (KBr, ν_{max}, cm^{-1}): 3286 (OH), 1672 (C=N), 1640 (C=C). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.79 (3H, s, CH_3-10), 0.84 (3H, s, CH_3-13), 4.04 (1H, br.s, Hβ-3), 5.35 (1H, m, H-11), 8.39 (br.s, CN–OH). ^{13}C NMR spectrum (δ, ppm): 17.3 (C-18), 17.6 (C-19), 66.9 (C–OH), 115.2 (C-11), 148.1 (C-9), 171.8 (C=N).

Antitumor Activity *in vitro*. Cell Culture. Cell line HeLa supplied by the National Laboratory of Cell Cultures of Piune University (India) was preserved as monolayers in square vials (75 cm^2) in ordinary Eagles medium with fetal bovine serum (10%), gentamycin (2 mg), penicillin (100 ED/mL), and streptomycin (100 μg/mL). Cells were grown in humidified incubators at 37°C with regulated addition of CO_2 (5%). Fresh medium was used every 5-6 d.

On the day of the experiment, cells were rinsed with phosphate buffered saline and separated from the vial surface by adding trypsin (0.25%) in EDTA buffer. Then, each well of a 96-well plate was charged with cell-culture suspension (250 μL, 5×10^3) and left overnight. Stock solutions of tested compounds were prepared under sterile conditions immediately before the experiment. Each compound was tested in two concentrations (10 and 100 μg/mL). The required amount was added to each well and left for 72 h. Each compound was tested against the corresponding control.

Each well was studied at the end of the incubation period by 40× magnification in an inverted microscope. The cytostatic effect of each concentration of tested compound toward the tumor cell line was determined.

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