Bioorganic & Medicinal Chemistry Letters 20 (2010) 5495–5498

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

[17(20)*E*]- and [17(20)*Z*]-pregna-5,17(20)-dien-21-oylamides. Facile synthesis and primary evaluation for cancer cells proliferation

Sergey V. Stulov [a], Maria G. Zavialova [a], Arif R. Mehtiev [a], Roman A. Novikov [b], Yaroslav V. Tkachev [b], Vladimir P. Timofeev [b,*], Alexander Yu Misharin [a]

[a] *V.N. Orekhovich Institute of Biomedical Chemistry RAMS, Moscow, Russia*
[b] *V.A. Engelhardt Institute of Molecular Biology RAS, Moscow, Russia*

ARTICLE INFO

Article history:
Received 17 June 2010
Revised 15 July 2010
Accepted 16 July 2010
Available online 21 July 2010

Keywords:
Pregnenolone derivatives
Synthesis
Cancer cells proliferation

ABSTRACT

Reaction of 17α-bromo-21-iodo-3β-acetoxypregn-5-en-20-one with ammonia, primary, and secondary amines is simple and convenient method for preparation of [17(20)*E*]- and [17(20)*Z*]-pregna-5,17(20)-dien-21-oylamides. Synthesis and characteristics of 12 related amides are presented. Primary testing on cells proliferation indicated differing effects of synthesized compounds on androgen insensitive MCF-7 cells and androgen sensitive LNCaP cells.

Steroid derivatives comprising ring D modified with nitrogen containing substituents exhibit a lot of different biological activities and are attractive for biomedicine. A large number of steroid derivatives containing imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, pyridyl, pyrimidyl, oxazinyl, tetrahydrooxazinyl, dihydrooxazinyl hetorocycles are known to efficiently inhibit activity of 17(*R*)-hydroxylase/17,20-lyase and used as potent agents for prostate cancer treatment.[1–3] Some 17-picolyl, 17-picolinylidene, N-oxy 17-picolyl and 17-picolinylideneandrost-5-ene derivatives inhibit activity of aromatase and exhibit antitumor activity against human breast adenocarcinoma, human melanoma and human prostate carcinoma cells.[4,5] Among the N- and C-substituted 1,3,5(10)-estratrien-[17,16-*c*]-pyrazoles potent inhibitors of 17β-hydroxysteroid-dehydrogenase type 1 were found.[6] 23-Azasterol analogs containing either amide, or amino group, inhibit ergosterol biosynthesis, Δ24-sterol methyltransferase activity, and exhibit anti-parasitic, anti-microbial, and anti-fungal properties.[7,8] Last decade some novel schemes for synthesis of biologically active nitrogen containing steroid derivatives have been developed starting from pregnenolone.[9–12]

Being involved in synthesis and studies of new steroid derivatives comprising nitrogen containing substituents in D ring, in this Letter, we report a new simple procedure for preparation of both [17(20)*E*]- and [17(20)*Z*]-isomeric pregna-5,17(20)-dien-21-oylamides, and results of primary testing of synthesized compounds on proliferation of human breast carcinoma MCF-7 cells and human prostate carcinoma LNCaP cells.

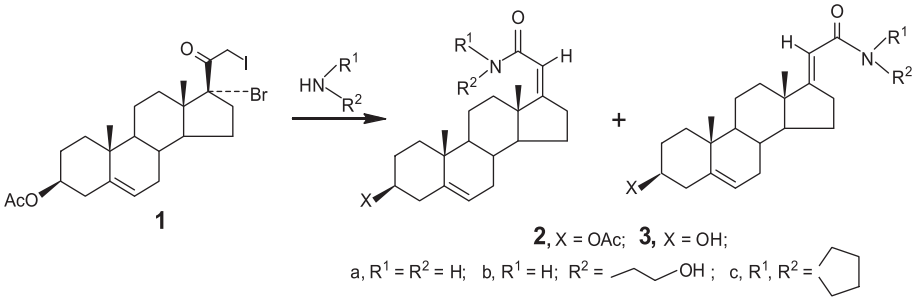
We discovered that known 17α-bromo-21-iodo-3β-acetoxypregn-5-en-20-one **1**, prepared from pregnenolone according to reported procedure,[13] smoothly reacts with ammonia, primary and secondary amines to give 3β-acetoxypegna-5,17(20)-dien-21-oylamides **2**, as mixtures of corresponding [17(20)*E*]- and [17(20)*Z*]-isomers. Three instances of this reaction, where ammonia, ethanolamine and pyrrolidine were used, are shown in Scheme 1; reaction conditions and yields are summarized in Table 1.[14]

This reaction resembles the known transformation of 20-ketopregnan-17,21-dihalohenides to corresponding pregn-17(20)-enoic acids in alkali medium,[15–17] occurring via Favorskii rearrangement. Reaction in benzene preferably yields [17(20)*E*]-isomer (configuration determination is discussed below), while reaction in more polar solvents such as acetonitrile, dioxane, methanol, etc., led to equimolar mixture of both [17(20)*E*]- and [17(20)*Z*]-isomers. The target amides **2(a–c)** (mixture of *E*- and *Z*-isomers) isolated by silica gel flash chromatography were then successfully separated by preparative TLC to give individual [17(20)*E*]- and [17(20)*Z*]-isomers.[18] Each isolated acetate **2** was transformed to related 3β-hydroxy amide **3**[19] in quantitative yield by treatment with K_2CO_3 in boiling aqueous methanol.

In order to determine the configuration of substituents at 17(20) double bond in target isomeric amides **3**(**a–c**), we performed a series of NMR NOESY experiments. Rigid structure of C-17 substituent, originating from the presence of 17(20) double bond, ensures very characteristic and strong NOE signals for

* Corresponding author. Tel.: +7 499 135 9859; fax: +7 499 135 1405.
E-mail address: tim@eimb.ru (V.P. Timofeev).

0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.07.075



Scheme 1.

Table 1
Synthesis of 3β-acetoxypregna-5,17(20)-dien-21-oylamides **2**(**a**–**c**)

Item	Amine	Reaction condition	Yield (%)	HRMS	17(20)*E*/17(20)*Z* ratio[a]
a	NH_3	35 equiv, dioxane–MeOH (1:1), rt, 6 h	42 (80[b])	Calcd for $[C_{23}H_{34}NO_3]$+: 372.2539; found: 372.2541	54:46
		70 equiv, MeOH, rt, 48 h	72%		49:51
b	H_2N—OH	3 equiv, benzene, reflux, 30 min	38		61:39
		5 equiv, dioxane, reflux, 15 min	56	Calcd for $[C_{25}H_{38}NO_4]$+: 416.2801; found: 416.2803	53:47
		5 equiv, CH_3CN, reflux, 15 min	61		52:48
c	HN	5 equiv, benzene, reflux, 30 min	52		66:34
		5 equiv, CH_3CN, reflux, 30 min	76	Calcd for $[C_{27}H_{40}NO_3]$+: 426.3008; found: 426.3012	50:50

[a] The ratio of *E*/*Z* isomers was calculated from integral intensities of characteristic resonances in ^{1}H NMR spectrum.
[b] The yield calculated on converted **1**.

H-20 protons in both [17(20)*Z*]- and [17(20)*E*]-amides **3**. In (*Z*)-isomers this proton is located closely to the H-16 protons, while in (*E*)-isomers it looks in the opposite side, in close vicinity to the H-12 protons. Figure 1 shows fragments of NOESY spectra of isomeric 3β-hydroxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamides (*Z*)-**3c** and (*E*)-**3c**. Interaction between H-20 and H-16 protons, and correspondingly arising NOESY cross-peaks at (5.69, 2.55) ppm, and (5.69, 2.30) ppm, shown in Figure 1a, unequivocally identify this compound as (*Z*)-**3c**. Similarly, related (*E*)-isomer (*E*)-**3c** was identified by the cross-peaks between H-20 and H-12 (5.69, 1.28)

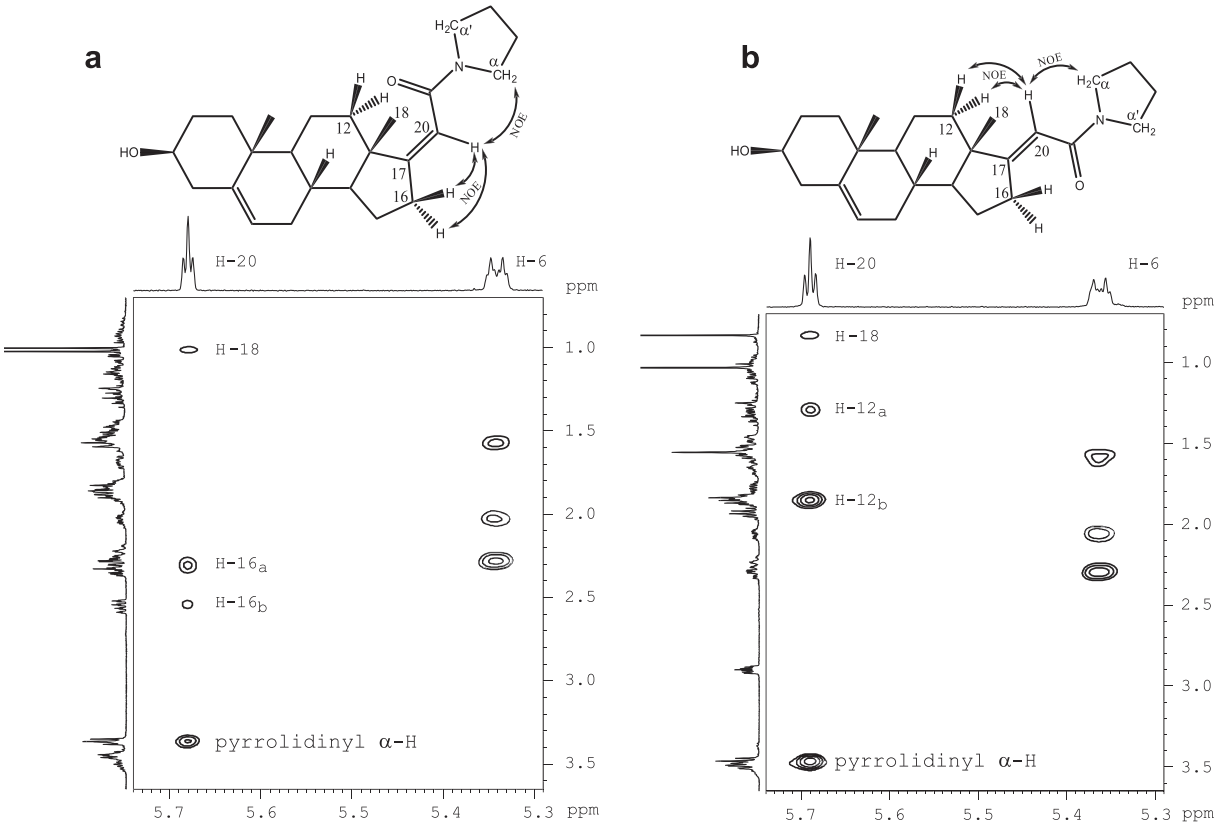


Figure 1. NOESY spectra of 3β-hydroxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamides (*Z*)-**3c** (a), and (*E*)-**3c** (b); interactions leading to characteristic cross-peaks are depicted by arrows in corresponding structure formula.

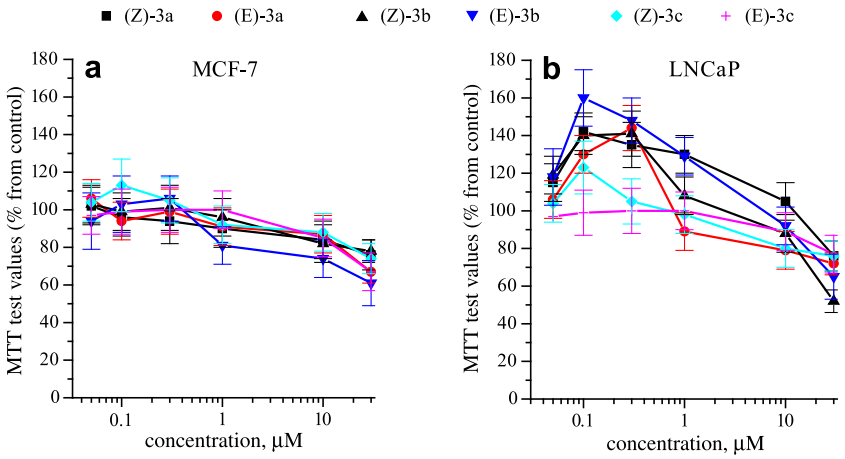


Figure 2. Effects of [17(20)*Z*]- and [17(20)*E*]-pregna-5,17(20)-dien-21-oylamides **3**(**a**–**c**) on proliferation of MCF-7 cells (a) and LNCaP cells (b).

ppm and (5.69, 1.81) ppm, as shown in Figure 1b. NOESY spectra of (*Z*)- and (*E*)-isomers of amides **3a** and **3b** reveal the very similar patterns.

The isomeric amides **2** and **3** display chemical shift differences of H-16, H-18, and H-20 resonances in ^{1}H NMR spectra,[18,19] and this was attributed to spatial arrangement of polar amide group. Among the each pair of isomeric amides **2**(**a**–**c**), the compound with higher chromatographic mobility exhibiting H-20 and H-18 resonances in lower field and separated resonances for two H-16 protons,[18,19] was considered to be (*Z*)-isomer, whilst the (*E*)-isomer had lower chromatographic mobility. The resonances of C-20 in ^{13}C NMR spectra of compounds (*Z*)-**2a**, (*Z*)-**2b** and (*Z*)-**2c**, compared to those of related (*E*)-isomers (*E*)-**2a**, (*E*)-**2b** and (*E*)-**2c**, were strongly downshifted (by 4–5 ppm),[18] that also confirms isomers identification. Obviously, aforementioned conclusion was also correct for related 3β-hydroxy amides **3**,[19] and this was completely confirmed by the NOESY studies described above.

Planning further studies of effects of 3β-hydroxypregna-5,17(20)-dien-21-oylamides **3**(**a**–**c**) on steroidogenesis and steroid responses in cultured cancer cells, we performed primary testing of these compounds on viability and proliferation in androgen insensitive human breast carcinoma MCF-7 cells and androgen sensitive human prostate carcinoma LNCaP cells.[20] Effects of compounds **3**(**a**–**c**) on cell proliferation were determined in serum free medium after incubation during 48 h by MTT assay, based on mitochondrial reduction of the yellow MTT tetrazolium dye to a highly colored blue formazan product.[21]

Results are shown in Figure 2. Compounds **3**(**a**–**c**) slightly decreased viability of MCF-7 cells at concentrations exceeding 30 μM (Fig. 2a); there were no significant differences in cytotoxicity of (*Z*)- and (*E*)-isomers. On the contrary, in LNCaP cells compounds **3a** and **3b** showed dual effects on proliferation: at a concentrations of 0.1, and 0.3 μM they considerably increase proliferation, while at a concentrations exceeding 30 μM an inhibiting effect was observed (Fig. 2b). It should be noted that similar increasing of proliferation of androgen sensitive cells by low doses of hormones, their analogs, and several drugs was repeatedly reported earlier.[22–25] [17(20)*Z*]-3β-Hydroxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamide (*Z*)-**3c** exhibited slight proliferative effect in LNCaP cells at concentration of 0.1 μM, the corresponding (*E*)-isomer (*E*)-**3c** did not increased proliferation of LnCaP cells at low concentrations.

In conclusion, obtained results indicated: (i) interaction of 17α-bromo-21-iodo-3β-acetoxypregn-5-en-20-one with ammonia and amines is simple and convenient approach for preparation of [17(20)*E*]- and [17(20)*Z*]-pregna-5,17(20)-dien-21-oylamides; (ii) ^{1}H NMR provides simple method for identification of corresponding (*E*)- and (*Z*)-isomers; (iii) synthesized 3β-hydroxypregna-5,17(20)-dien-21-oylamides displayed differing effects on proliferation of androgen insensitive MCF-7 cells and androgen sensitive LNCaP cells, their antiproliferative activity was substantially lower than that of reported earlier pregnane derivatives comprising nitrogen containing heterocycles.[5,11,12]

Acknowledgments

This study was supported by Russian Foundation for Basic Research (RFBR grants Nos. 09-04-00454-a and 10-04-90044-Bel_a) and the Molecular and Cellular Biology Program of Russian Academy of Sciences.

References and notes

1. Njar, V. C.; Brodie, A. M. *Curr. Pharm. Des.* **1999**, *5*, 163. and the references therein.
2. Wolfling, J.; Hackler, L.; Mernyrak, E.; Schneider, G.; Troth, I.; Szrecsi, M., et al *Steroids* **2004**, *69*, 451.
3. Wolfling, J.; Oravecz, E. A.; Ondrer, D.; Mernyrak, E.; Schneider, G.; Troth, I.; Szrecsi, M.; Julesz, J. *Steroids* **2006**, *71*, 809.
4. Penov-Gaši, K.; Stanković, S.; Csanadi, J.; Djurendić, E.; Sakač, M.; Medić-Mijačević, L.; Arcson, O.; Stojanović, S.; Andrić, S.; Molnar Gabor, D.; Kovačević, R. *Steroids* **2001**, *66*, 645.
5. Penov-Gaši, K.; Djurendić-Brenesel, M.; Djurendić, E.; Sakač, M.; Csanádi, J.; Daljev, J.; Armbruster, T.; Andrić, S.; Sladić, D.; Božić, T.; Novaković, I.; Juranić, Z. *Steroids* **2007**, *72*, 31.
6. Fischer, D. S.; Allan, G. M.; Bubert, C.; Vicker, N.; Smith, A.; Tutill, H. J.; Purohit, A.; Wood, L.; Packham, G.; Mahon, M. F.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2005**, *48*, 5749.
7. Gros, L.; Lorente, S. O.; Jimenez, C. J.; Yardley, V.; Rattray, L.; Wharton, H.; Little, S.; Croft, S. L.; Ruiz-Perez, L. M.; Gonzalez-Pacanowska, D.; Gilbert, I. H. *J. Med. Chem.* **2006**, *49*, 6094.
8. Renard, D.; Perruchon, J.; Giera, M.; Müller, J.; Bracher, F. *Bioorg. Med. Chem.* **2009**, *17*, 8123.
9. Magaraci, F.; Jimenez, C. J.; Rodrigues, C.; Rodrigues, J. C. F.; Braga, M. V.; Yardley, V.; de Luca-Fradley, K.; Croft, S. L.; de Souza, W.; Ruiz-Perez, L. M.; Urbino, J.; Gonzalez-Pacanowska, D.; Gilbert, I. H. *J. Med. Chem.* **2003**, *46*, 4714.
10. Zavarzin, I. V.; Kamernitsky, A. V.; Chertkova, V. V.; Chernoburova, E. I.; Yarovenko, V. N.; Kraushkin, M. M.; Kachala, V. V. *Arkivoc* **2008**, 62.
11. Banday, A. H.; Mir, B. P.; Khazir, J.; Suri, K. A.; Kumar H. M. S. *Steroids* **2010**, doi:10.1016/j.steroids.2010.02.014.
12. Banday, A. H.; Verma, M.; Srikakulam, S.; Gupta, B. D.; Kumar, H. M. S. *Steroids* **2010**. doi:10.1016/j.steroids.2010.02.015.
13. Julian, P. L.; Karpel, W. J. *J. Am. Chem. Soc.* **1950**, *72*, 362.
14. Reagents and solvents were purchased from 'Aldrich', 'Merck', and 'Acros'; pregnenolone—from 'MP Biomedicals'; culture plastics—from 'Greiner', 'Costar' and 'Corning'; culture media and fetal calf serum—from 'Gibco BRL' and 'HyClone'; phosphate buffered saline and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)—from 'Sigma'. HRMS were registered on a Bruker 'Apex Ultra' FT ICR MS instrument at ion positive electro spray ionization mode; ^{1}H NMR and ^{13}C NMR spectra—on an AMX-III instrument (Bruker, 400 MHz) in $CDCl_3$; the values of δ for ^{1}H in $CHCl_3$ was 7.25 ppm and ^{13}C in $CDCl_3$ was 77.16 ppm.

15. Marker, R.; Crooks, H.; Wagner, R. *J. Am. Chem. Soc.* **1942**, *64*, 1276.
16. Romo, J.; Romo de Vivar, A. *J. Am. Chem. Soc.* **1957**, *79*, 1118.
17. Oliveto, E. P.. In *Organic Reactions in Steroid Chemistry*; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold Company, 1972; Vol. II, pp 176–179.
18. Isomeric [17(20)*Z*]- and [17(20)*E*]-acetates were separated by TLC on UV_{254}-HPTLC silica gel plates from 'Merck' in an appropriate solvent systems. *[17(20)Z]-3β-Acetoxypregna-5,17(20)-dien-21-oylamide* (*Z*)-**2a** [HRMS is given in Table 1; ^{1}H NMR: 1.03 (6H, s, coincided H-18 and H-19); 2.02 (3H, s, Ac); 2.50–2.70 (1H, m, H-16); 4.59 (1H, m, H-3); 5.23 (1H, br, CONH); 5.38 (1H, m, H-6); 5.64 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 16.20; 19.30; 21.04; 21.43; 23.85; 27.78; 29.72; 31.52; 31.82; 32.92; 33.97; 36.96; 38.15; 45.42; 50.02; 56.47; 73.94; 113.19; 122.20; 140.02; 166.56; 168.67; 170.54] and *[17(20)E]-3β-Acetoxypregna-5,17(20)-dien-21-oylamide* (*E*)-**2a** [HRMS is given in Table 1; ^{1}H NMR: 0.83 (3H, s, H-18); 1.04 (3H, s, H-19); 2.02 (3H, s, Ac); 2.87 m (2H, m, H-16); 4.60 (1H, m, H-3); 5.21 (1H, br, CONH); 5.38 (1H, m, H-6); 5.48 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 18.45; 19.44; 21.06; 21.49; 24.66; 27.86; 30.03; 31.70; 31.78; 35.46; 36.82; 37.11; 38.20; 45.96; 50.33; 53.83; 73.92; 109.80; 122.41; 139.82; 169.13; 170.57; 172.81] were separated in hexane–EtOAc (1:1) mixture; *[17(20)Z]-3β-acetoxypregna-5,17(20)-dien-21-oyl-N-(2-hydroxyethyl)-amide* (*Z*)-**2b** [HRMS is given in Table 1; ^{1}H NMR: 1.02 (6H, s, coincided H-18 and H-19); 2.02 (3H, s, Ac); 2.50–2.66 (1H, m, H-16); 3.42 and 3.73 (each 2H, br m, NCH_2 and OCH_2); 4.60 (1H, m, H-3); 5.38 (1H, m, H-6); 5.63 (1H, t, *J* = 2.0 Hz, H-20); 5.81 (1H, br, CONH); ^{13}C NMR: 16.44; 19.36; 21.12; 21.49; 23.94; 27.85; 29.78; 31.58; 31.86; 32.87; 34.21; 36.77; 37.02; 38.21; 42.69; 45.42; 50.05; 56.49; 62.93; 74.00; 114.01; 122.27; 140.06; 165.59; 168.30; 170.62] and *[17(20)E]-3β-acetoxypregna-5,17(20)-dien-21-oyl-N-(2-hydroxyethyl)-amide* (*E*)-**2b** [HRMS is given in Table 1; ^{1}H NMR: 082 (3H, s, H-18); 1.04 (3H, s, H-19); 2.02 (3H, s, Ac); 2.88 (2H, m, H-16); 3.45 and 3.74 (each 2H, br m, NCH_2 and OCH_2); 4.60 (1H, m, H-3); 5.38 (1H, m, H-6); 5.44 (1H, t, *J* = 2.0 Hz, H-20); 5.82 (1H, br, CONH); ^{13}C NMR:18.48; 19.43; 21.06; 21.49; 24.69; 27.86; 30.00; 31.70; 31.78; 35.50; 36.82; 37.11; 38.19; 42.59; 45.89; 50.33; 53.87; 63.14; 73.93; 110.33; 122.43; 139.81; 168.78; 170.60; 171.92] were separated in CH_2Cl_2–acetone (2:1) mixture; *[17(20)Z]-3β-acetoxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamide* (*Z*)-**2c** [HRMS is given in Table 1; ^{1}H NMR: 1.02 and 1.03 (each 3H, s, H-18 and H-19); 2.02 (3H, s, Ac); 2.50–2.60 (1H, m, H-16); 3.37 and 3.42 (each 2H, m, CH_2 in pyrrolidine moiety); 4.59 (1H, m, H-3); 5.36 (1H, m, H-6); 5.68 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 16.56; 19.36; 21.05; 21.49; 23.97; 24.61; 26.14; 27.85; 31.62; 31.87; 31.89; 34.10; 36.78; 37.04; 38.21; 45.07; 45.18; 47.57; 50.17; 56.28; 74.01; 114.39; 122.33; 140.04; 161.40; 166.91; 170.63] and *[17(20)E]-3β-acetoxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamide* (*E*)-**2c** [HRMS is given in Table 1; ^{1}H NMR: 0.83 (3H, s, H-18); 1.04 (3H, s, H-19); 2.02 (3H, s, Ac); 2.89 (2H, m, H-16); 3.48 (4H, m, CH_2 in pyrrolidine moiety); 4.50 (1H, m, H-3); 5.35 (1H, m, H-6); 5.69 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 18.55; 19.40; 21.05; 21.45; 24.47; 24.64; 26.30; 27.82; 29.96; 31.70; 31.76; 35.67; 36.78; 37.06; 38.15; 45.46; 45.78; 46.72; 50.36; 53.94; 73.91; 108.94; 122.46; 139.74; 166.42; 170.55; 171.19] were separated in hexane–acetone (3:1) mixture.
19. *[17(20)Z]-3β-Hydroxypregna-5,17(20)-dien-21-oylamide* (*Z*)-**3a** (HRMS, calcd for $C_{21}H_{32}NO_2^+$: 330.2433; found: 330.2437; ^{1}H NMR: 1.01 and 1.02 (each 3H, s, H-18 and H-19); 2.50–2.70 (1H, m, H-16); 3.51 (1H, m, H-3); 5.26 (1H, br, CONH); 5.35 (1H, m, H-6); 5.65 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 16.30; 19.45; 21.15; 23.92; 29.79; 31.63; 31.76; 31.89; 32.96; 34.11; 36.69; 37.28; 42.38; 50.16; 56.59; 71.86; 113.30; 121.40; 141.24; 166.62; 168.83); *[17(20)E]-3β-Hydroxypregna-5,17(20)-dien-21-oylamide* (*E*)-**3a** (HRMS, calcd for $C_{21}H_{32}NO_2^+$: 330.2433; found: 330.2430; ^{1}H NMR: 0.83 (3H, s, H-18); 1.03 (3H, s, H-19); 2.87 m (2H, m, H-16); 3.52 (1H, m, H-3); 5.21 (1H, br, CONH); 5.35 (1H, m, H-6); 5.48 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 18.45; 19.53; 21.11; 24.67; 29.79; 30.09; 31.74; 31.77; 35.50; 36.73; 37.38; 42.38; 46.02; 50.42; 53.91; 71.78; 109.68; 121.47; 140.90; 169.20; 173.25); *[17(20)Z]-3β-hydroxypregna-5,17(20)-dien-21-oyl-N-(2-hydroxyethyl)-amide* (*Z*)-**3b** (HRMS, calcd for $C_{23}H_{36}NO_3^+$: 374.2695; found: 374.2701; ^{1}H NMR: 1.01 and 1.02 (each 3H, s, H-18 and H-19); 2.50–2.66 (1H, m, H-16); 3.45 and 3.74 (each 2H, m, NCH_2 and OCH_2); 3.50 (1H, m, H-3); 3.73 (2H, br t. *J* = Hz, OCH_2); 5.35 (1H, m, H-6); 5.63 (1H, t, *J* = 2.0 Hz, H-20); 5.81 (1H, br, CONH); ^{13}C NMR: 16.49; 19.45; 21.17; 23.94; 29.78; 31.63; 31.75; 31.87; 32.83; 34.28; 36.68; 37.27; 42.36; 42.66; 50.13; 56.54; 62.89; 71.84; 114.01; 121.34; 141.14; 165.45; 168.35); *[17(20)E]-3β-hydroxypregna-5,17(20)-dien-21-oyl-N-(2-hydroxyethyl)-amide* (*E*)-**3b** (HRMS, calcd for $C_{23}H_{36}NO_3^+$: 374.2695; found: 374.2691; ^{1}H NMR: 082 (3H, s, H-18); 1.03 (3H, s, H-19); 2.89 (2H, m, H-16); 3.46 and 3.74 (each 2H, m, NCH_2 and OCH_2); 3.51 (1H, m, H-3); 3.74 (2H, br t. *J* = Hz, OCH_2); 5.35 (1H, m, H-6); 5.44 (1H, t, *J* = 2.0 Hz, H-20); 5.79 (1H, br, CONH); ^{13}C NMR: 14.25; 18.55; 19.59; 21.19; 24.76; 29.85; 30.08; 31.81; 31.86; 35.62; 36.79; 37.44; 42.45; 42.67; 50.51; 54.02; 63.26; 71.86; 110.36; 121.57; 140.95; 164.97; 168.87); *[17(20)Z]-3β-hydroxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamide* (*Z*)-**3c** (HRMS, calcd for $C_{25}H_{38}NO_2^+$: 384.2903; found: 384.2902; ^{1}H NMR: 1.01 and 1.03 (each 3H, s, H-18 and H-19); 2.50–2.60 (1H, m, H-16); 3.37 and 3.42 (each 2H, m, CH_2 in pyrrolidine moiety); 3.60 (1H, m, H-3); 5.35 (1H, m, H-6); 5.68 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 16.64; 19.52; 21.18; 24.05; 24.68; 26.21; 31.74; 31.82; 31.96; 31.98; 34.25; 36.77; 37.38; 42.46; 45.16; 45.24; 47.64; 50.33; 56.40; 71.92; 114.44; 121.45; 141.22; 161.45; 166.95); *[17(20)E]-3β-hydroxypregna-5,17(20)-dien-21-oyl-pyrrolidinylamide* (*E*)-**3c** (HRMS, calcd for $C_{25}H_{38}NO_2^+$: 384.2903; found: 384.2912; ^{1}H NMR: 0.83 (3H, s, H-18); 1.03 (3H, s, H-19); 2.90 (2H, m, H-16); 3.49 (5H, m, overlapped H-3 and CH_2 in pyrrolidine moiety); 5.37 (1H, m, H-6); 5.69 (1H, t, *J* = 2.0 Hz, H-20); ^{13}C NMR: 18.68; 19.60; 21.22; 24.58; 24.77; 26.42; 30.08; 31.84; 31.86; 31.87; 38.84; 36.80; 37.45; 42.48; 45.54; 45.90; 46.80; 50.59; 54.14; 71.87; 109.04; 121.64; 140.94; 166.50; 171.31).
20. Human breast carcinoma MCF-7 cells and human prostate carcinoma LNCaP cells (obtained from ATCC) were cultured in a 96-well plates at 37 °C in an atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal calf serum. Before the experiments, cells were incubated for 24 h in a serum free medium. The tested compounds at were added to the culture medium in ethanolic solutions.
21. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55. Cells were incubated with compounds tested for 48 h at 37 °C in serum free medium. Then the medium was aspirated, 125 μl of MTT solution in phosphate buffered saline (1 mg /ml) was added to each well and plates were incubated at 37 °C for 4 h more, thereafter 125 μl of stop solution (0.1 M HCl in *i*PrOH containing 10% Triton X100) was added to each well, and the plates were stored overnight at room temperature. The absorbance at 630 nm in each well was measured on a LKB microplate reader. The values for each point were calculated from six wells; all experiments were carried out in triplicate; toxicity of compounds tested was calculated from plot: cell viability (% from control) versus concentration of compounds tested in medium.
22. Benz, C.; Hollander, C.; Miller, B. *Cancer Res.* **1986**, *46*, 2276.
23. Baratta, M.; Grolli, S.; Poletti, A.; Ramoni, R.; Motta, M.; Tamanini, C. *J. Endocrinol.* **2000**, *167*, 53.
24. Migliaccio, A.; Castoria, G.; Di Domenico, M.; de Falco, A.; Bilancio, A.; Lombardi, M.; Barone, M. V.; Ametrano, D.; Zanini, M. S.; Abbondanza, C.; Auricchio, F. *EMBO J.* **2000**, *19*, 5406.
25. Hara, T.; Miyazaki, J.-I.; Araki, H.; Yamaoka, M.; Kanzaki, N.; Kusaka, M.; Miyamoto, M. *Cancer Res.* **2003**, *63*, 149.