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SYNTHESIS AND INTRAMOLECULAR CYCLIZATION OF 2,3-SECO-LUPANE TRITERPENOIDS WITH AN ETHYLKETONE FRAGMENT

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A 1-cyano-2,3-secolupane derivative with an ethylketone fragment was synthesized from 2-hydroxyoximodihydrobetulonic acid methyl ester via a Grignard reaction followed by a Beckmann rearrangement and was used further to prepare α*-bromo-substituted diastereomers. Nitrile-anionic cyclization of the ethylketone and* α*-bromo isomers produced cyclic derivatives with an alkenenitrile and an* α,β*-unsaturated ketone in ring A, respectively. The obtained compounds did not exhibit cytotoxic activity according to biological screening data.*

Keywords: A-secotriterpenoids, dihydrobetulonic acid, Beckmann rearrangement, nitrile-anionic cyclization, ethylketone, cytotoxic activity.

Reductive allyl(alkyl)ation of triterpenoids has been achieved a few times, indicating that this approach could be used successfully to synthesize new biologically active compounds. For example, C-3 allylation under Grignard reaction conditions was reported and helped to improve the antidiabetic, cytotoxic, and anti-inflammatory activity of triterpenoids [1–5], including betulinic acid. Previously, 3-alkyl-substituted triterpenoids were shown by us to be useful as intermediates for preparing bioactive triterpene derivatives with a fragmented or five(six)-membered ring A [6–10], including lupane derivatives with pronounced antitumor properties such as methyl 3-(1-bromoethyl)-3-oxo-1-cyano-2,3-seco-2-norlup-20(29)-en-30-al-28-oate and methyl 1-cyano-3-ethyl-2-norlup-1(3),20(29)-dien-30-al-28-oate [10]. New fragmented and cyclic 3-ethyl-substituted lupane-type derivatives were synthesized and their cytotoxic activity was evaluated in continuation of research on the structure–activity (biological) relationship of C-3 alkylated triterpenoids.

The starting material for preparing the C-3 ethyl-substituted triterpenoids was hydroxyiminoketone **1**, which was prepared by sequential transformations from 20,29-dihydrogenated betulin [11]. The corresponding 3β-hydroxy-3α-ethyl derivative **2** was prepared using a Grignard reaction with C_2H_5MgBr as the alkylating agent [9, 10]. The IR spectrum of **2** exhibited bands for C=N (1644 cm^{-1}) and OH stretching vibrations (3313, 3437 cm^{-1}). The ^1H NMR spectrum of **2** contained characteristic doublets for the methylene H-1 protons at δ 1.52 and 3.38 ppm (AB-system, J = 12.6 Hz); a broad singlet for the OH at δ 5.89 ppm; and a resonance for the methylene H-32 protons as a doublet of quartets with centers at 1.76 and 1.85 ppm (J = 13.8, 7.2 Hz). The ^{13}C NMR spectrum of **2** displayed resonances for C atoms bonded to OH (79.59 ppm) and hydroxyimine groups (162.63 ppm).

The ethyl derivative **2** underwent a Beckmann rearrangement using $SOCl_2$–CH_2Cl_2 to form the corresponding 2,3-seco-derivative **3**. The IR spectrum of **3** had characteristic absorption bands for C=O (1695 cm^{-1}) and C≡N (2234). The ^1H NMR spectrum of seco-derivative **3** exhibited two doublets for the methylene H-1 protons with centers at 2.34 and 2.54 ppm (AB-system, J = 18.0 Hz) and two types of resonances for the ethyl moiety, i.e., a triplet for the methyl at δ 1.08 ppm (J = 7.2 Hz) and a doublet of quartets at δ 2.55 and 2.74 ppm (J = 19.6 and 7.2 Hz). The ^{13}C NMR spectrum of **3** included characteristic resonances of cyano and keto C atoms at δ 118.55 and 216.82 ppm, respectively.

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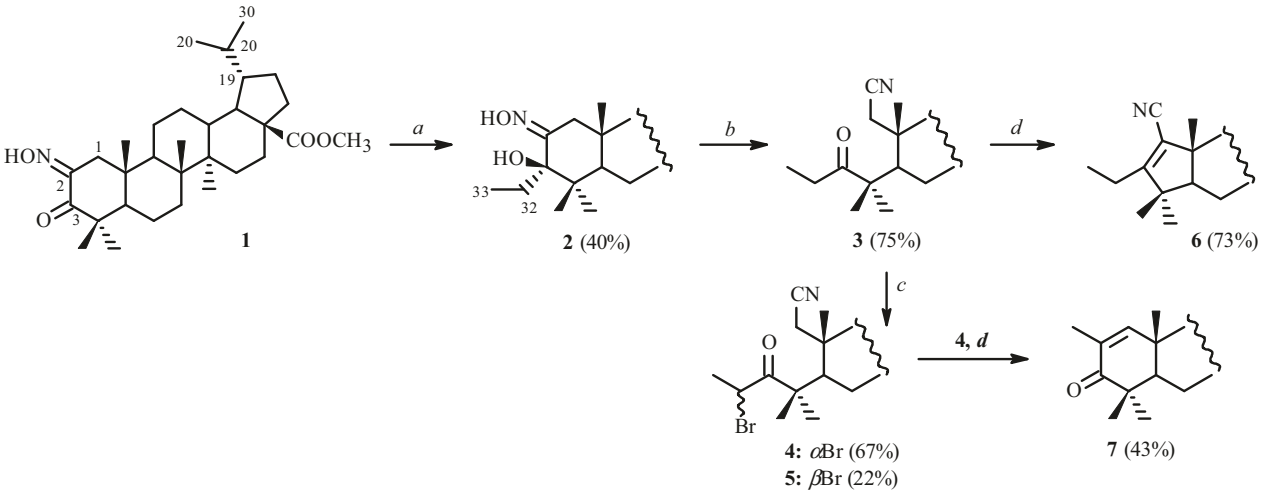

a. C_2H_5MgI–$(C_2H_5)_2O$–THF, 2 h; *b*. $SOCl_2$–CH_2Cl_2, 30 min; *c*. $C_5H_6Br_3N$–CH_3COOH, 6 h; *d*. *t*-BuOK–*t*-BuOH, 2 h.

Scheme 1

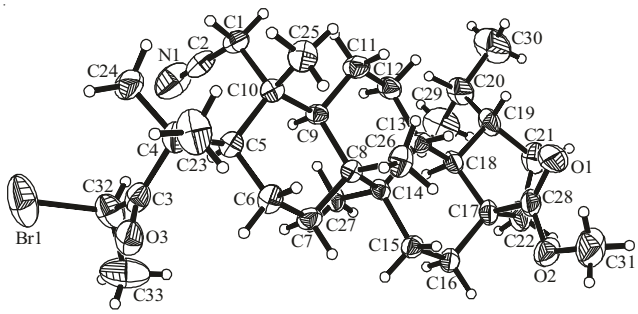

Fig. 1. General view of methyl (3*R*)-3-(1-bromoethyl)-3-oxo-1-cyano-2,3-seco-2-norlup-28-oate (**4**) from an XSA with 30% probability thermal ellipsoids.

The ethylketone of 2,3-seco-derivative **3** was activated by halogenation (Scheme 1) using the brominating agent pyridinium bromide perbromide [7, 9, 10]. The reaction proceeded with heating and stirring. Pure reaction products were isolated by column chromatography and included isomeric bromo-substituted derivatives **4** and **5**, the NMR spectra of which differed slightly. For example, ^{1}H NMR spectra of **4** and **5** showed the protons of the bromo-substituted ethyl group as a doublet for the methyl at 1.74 ppm (J = 6.6 Hz). The methine H-32 proton resonated as a quartet with SSCC 6.6 Hz at 5.04 (**4**) or 5.03 (**5**). Also, doublets for the methylene H-1 protons of **5** (2.58 and 2.69 ppm, J = 18.0 Hz) as compared to those of **4** (2.60 and 2.71 ppm, J = 17.9 Hz) appeared at stronger field. The ^{13}C NMR spectrum contained resonances at stronger field for the nitrile and ester of bromo derivative **4** at 118.58 (C-2) and 176.74 (C-28) as compared to the analogous resonances of **5** at 118.72 (C-2) and 176.76 ppm (C-28). The absolute configuration of asymmetric C-32 in diastereomers **4** and **5** was confirmed based on an X-ray crystal structure analysis (XSA) of **4** (Fig. 1).

Ethylketone **3** and bromo derivative **4** underwent intramolecular nitrile-anion cyclization [12] in the basic system *t*-BuOK–*t*-BuOH [13]. The cyclization product of **3** was α,β-alkenenitrile **6**. Its IR spectrum showed characteristic absorptions at 1594 (C=C), 1729 (C=O), and 2206 cm^{-1} (C≡N). The ^{1}H NMR spectrum of α,β-alkenenitrile **6** lacked doublets for the methylene H-1 protons of the starting compound and retained the characteristic resonances of the ethyl moiety as a triplet for the Me-32 protons with a center at 1.13 ppm (J = 7.6 Hz) and a quartet for the methylene H-31 protons with a center at 2.28 ppm (J = 7.6 Hz). The ^{13}C NMR spectrum of **6** exhibited resonances for the nitrile (118.33 ppm) and double-bond C atoms (120.52 and 172.41).

Cyclization of bromo derivative **4** occurred with loss of the nitrile, which led to formation of an α,β-unsaturated ketone in six-membered ring A of **7**. The IR spectrum of **7** lacked bands for the nitrile and exhibited a carbonyl absorption band

at 1667 cm^{-1}. The ^1H NMR spectrum included two characteristic singlets corresponding to the C-3 methyl protons (1.73 ppm) and olefinic H-1 proton (6.84 ppm). The ^{13}C NMR spectrum exhibited resonances for C-2 and C-1 of the double bond at 130.95 and 154.85 ppm, respectively, and for the carbonyl C atom at 205.62 ppm.

A study of the cytotoxic activity against eight tumor cell lines found that compounds **3**–**6** were nontoxic (IC_{50} > 100 μM). Thus, the C-30 aldehyde group played a decisive role in the manifestation of cytotoxic activity by the previously reported ethyl-substituted lupane derivatives [10].

EXPERIMENTAL

^1H NMR, ^{13}C NMR, and DEPT spectra of the synthesized compounds were recorded in $CDCl_3$ solutions (DMSO-d_6 was also used for **2**) with TMS internal standard on a Bruker Avance II NMR spectrometer (400 and 100 MHz, respectively). IR spectra were recorded from thin films prepared by evaporating $CHCl_3$ solutions of the compounds on a Bruker IFS 66/S FT-IR spectrometer (Germany). The threshold melting point at heating rate 1°C/min was determined on an OptiMelt MPA100 apparatus (USA). Specific optical rotation was measured in $CHCl_3$ solutions of the compounds on a PerkinElmer model 341 polarimeter at 589 nm. Elemental analyses (C, H, N) were performed in a Vario EL cube analyzer (Germany). The course of reactions was monitored by TLC on Sorbfil plates (Russia) after treatment with H_2SO_4 (5%) followed by heating at 95–100°C for 2–3 min. Column chromatography used Macherey-Nagel silica gel (60–200 μm) with elution by petroleum ether–EtOAc mixtures, the ratio of which was selected individually for each reaction product.

The XSA of **4** was performed on an Xcalibur Ruby single-crystal diffractometer (Agilent Technologies) by the standard method [Mo Kα-radiation, 295(2) K, ω-scanning in 1° steps]. Absorption corrections were applied empirically using the SCALE3 ABSPACK algorithm [14]. The structure was solved using the SHELXT program [15] and refined by anisotropic full-matrix least-squares methods over F^2 for all nonhydrogen atoms using the SHELXL program [16] and the OLEX2 graphics interface [17]. H atoms were refined using a rider model. The crystal ($C_{33}H_{52}BrNO_3$, MM 590.66) was monoclinic, space group $P2_1$, a = 7.705(2), b = 17.834(5), c = 11.818(3) Å, β = 92.74(2)°, V = 1621.9(7) $Å^3$, Z = 2, d_{calcd} = 1.209 g/cm^3, μ = 1.298 mm^{-1}. The final refinement parameters were R_1 0.0677 [for 2890 reflections with $I > 2\sigma(I)$], wR_2 0.1694 (for all 5882 independent reflections, R_{int} 0.0297), S = 1.035, Flack parameter 0.018(8). Results for the XSA of **4** were deposited in the Cambridge Crystallographic Data Centre under No. CCDC 2190242 and can be requested at www.ccdc.cam.ac.uk/data_request.cif.

Anhydrous solvents were prepared by standard methods [18]. Ketoxime **1** was prepared according to the literature method [11].

Preparation of Methyl 3β-Hydroxy-2-hydroxyimino-3α-ethyllup-28-oate (2). A solution of **1** (2.5 mmol) in a mixture of anhydrous Et_2O and THF (2:1, 14 mL) was added dropwise to a freshly prepared solution of C_2H_5MgBr (5.0 mmol) in anhydrous Et_2O (5 mL). The mixture was stirred with heating (60°C) for 2 h, cooled, worked up dropwise with ice water (25 mL) and HCl solution (20 mL, 17–20%), and stirred until the precipitate was fully dissolved. The resulting solution was extracted with EtOAc (3 × 30 mL). The organic layer was separated; washed with saturated $Na_2S_2O_3$ solution, $NaHCO_3$ solution (5%), and a small amount of H_2O; and dried over anhydrous $MgSO_4$. The EtOAc was evaporated in a rotary evaporator. The dry solid was purified by column chromatography (CC) with elution by petroleum ether–EtOAc (15:1). Colorless crystals, mp 206.4°C (hexane–EtOAc), yield 40%, R_f 0.21 (hexane–EtOAc, 5:1), $[\alpha]_D^{25}$ +1.7° (c 0.4, $CHCl_3$). IR (ν, cm^{-1}): 1644 (C=N), 1722 ($COOCH_3$), 3313 (OH), 3437 (NOH). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.73–0.78 (9H, m, H-29, 30, 33), 0.84, 0.86, 0.90 (3H each, s, CH_3), 0.97 (6H, s, CH_3), 1.52, 3.38 (1H each, d, J = 12.6, H-1, AB system), 1.76, 1.85 (1H each, dq, J = 14.3, 7.3, H-32), 2.19–2.27 (2H, m, H-19, 20), 3.64 (3H, s, H-31), 5.89 (1H, br.s, OH). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.64 (3H, t, J = 7.4, H-33), 0.69, 0.76 (3H each, d, J = 7.2, H-29, 30), 0.84 (3H, s, CH_3), 0.86 (6H, s, CH_3), 0.88, 0.99 (3H each, s, CH_3), 10.64 (1H, s, NOH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 7.38, 14.65, 14.74, 15.74, 16.86, 18.89, 19.02, 21.28, 22.76, 22.93, 23.98, 26.71, 26.78, 29.73, 32.08, 34.34, 36.62, 37.29, 38.07, 41.17, 41.23, 41.63, 42.67, 44.19, 45.13, 48.94, 50.28, 51.17, 53.10, 57.01, 79.59 (C-3), 162.63 (C-2), 176.88 (C-28). Found, %: C, 74.43; H, 10.52; N, 2.75. $C_{33}H_{55}NO_4$. Calcd, %: C, 74.81; H, 10.46; N, 2.64.

Synthesis of Methyl 3-Oxo-1-cyano-3-ethyl-2,3-seco-2-norlup-28-oate (3). A solution of **2** (1 mmol) in anhydrous CH_2Cl_2 (50 mL) was stirred and treated with $SOCl_2$ (3 mmol). The mixture was stirred for 30 min at room temperature. The solvent was evaporated in a rotary evaporator. Traces of $SOCl_2$ were removed by rinsing the precipitate twice with CH_2Cl_2.

The dry solid was purified by CC with elution by petroleum ether–EtOAc (15:1). Yield 75%, R_f 0.34 (hexane–EtOAc, 5:1), colorless crystals, mp 164.7°C (hexane–EtOAc), $[\alpha]_D^{25}$ –1.7° (*c* 0.4, $CHCl_3$). IR (ν, cm^{-1}): 1695 (C=O), 1725 ($COOCH_3$), 2234 (C≡N). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.75, 0.85 (3H each, d, J = 6.8, CH_3), 0.91, 0.95, 1.01, 1.16, 1.22 (3H each, s, CH_3), 1.08 (3H, t, J = 7.2, H-33), 2.19–2.30 (2H, m, H-19, 20), 2.34, 2.54 (1H, d, J = 18.0, H-1, AB system), 2.55, 2.74 (2H, dq, J = 19.6, 7.2, H-32), 3.63 (3H, s, H-31). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 8.71, 14.60, 14.67, 15.74, 18.78, 21.57, 21.82, 22.75, 22.91, 24.00, 24.07, 26.90, 29.49, 29.69, 29.71, 30.37, 31.82, 33.41, 37.18, 38.14, 40.55, 42.40, 43.00, 44.12, 45.06, 48.75, 48.88, 51.13, 52.87, 57.01, 118.55 (C-2), 176.76 (C-28), 216.82 (C-3). Found, %: C, 77.03; H, 10.38; N, 2.80. $C_{33}H_{53}NO_3$. Calcd, %: C, 77.45; H, 10.44; N, 2.74.

Method for Preparing 4 and 5. A solution of 2,3-seco-derivative **3** (0.4 mmol) in AcOH (30 mL) was stirred and treated with $C_5H_6Br_3N$. The reaction mixture was left on a magnetic stirrer with heating (115°C) for 6 h, diluted with H_2O, and extracted with EtOAc (3 × 20 mL). The organic layer was washed with $NaHCO_3$ solution (5%) and H_2O and dried over anhydrous $MgSO_4$. The solvent was evaporated in a rotary evaporator. The dry solid was purified by CC with elution by petroleum ether–EtOAc (15:1).

Methyl (3*R*)-3-(1-Bromoethyl)-3-oxo-1-cyano-2,3-seco-2-norlup-28-oate (4). Yield 67%, R_f 0.43 (hexane–EtOAc, 5:1, double elution), colorless crystals, mp 172.3°C (hexane–EtOAc), $[\alpha]_D^{22}$ +38.5° (*c* 0.5, $CHCl_3$). IR (ν, cm^{-1}): 1718 ($COOCH_3$, C=O), 2239 (C≡N). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.75, 0.85 (6H, d, J = 6.8, CH_3), 0.91, 0.98, 1.00, 1.23, 1.50 (3H each, s, CH_3), 1.74 (3H, d, J = 6.6, H-33), 2.19–2.32 (2H, m, H-19, 20), 2.60, 2.71 (1H, d, J = 17.9, H-1), 3.63 (3H, s, H-31), 5.04 (1H, q, J = 6.6, H-32). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.51, 14.67, 15.93, 18.95, 21.29, 21.92, 22.20, 22.41, 22.74, 22.92, 25.85, 26.99, 29.65, 29.73, 30.02, 31.78, 33.14, 37.16, 38.18, 40.34, 40.59, 42.64, 42.98, 44.10, 45.25, 46.01, 48.72, 51.16, 54.33, 57.00, 118.58 (C-2), 176.74 (C-28), 208.51 (C-3). Found, %: C, 66.83; H, 8.98; N, 2.50. $C_{33}H_{52}BrNO_3$. Calcd, %: C, 67.10; H, 8.87; N, 2.37.

Methyl (3*S*)-3-(1-Bromoethyl)-3-oxo-1-cyano-2,3-seco-2-norlup-28-oate (5). Yield 22%, R_f 0.34 (hexane–EtOAc, 5:1, double elution), colorless crystals, mp 201.8°C (hexane–EtOAc), $[\alpha]_D^{25}$ +5.7° (*c* 0.4, $CHCl_3$). IR (ν, cm^{-1}): 1718 ($COOCH_3$, C=O), 2237 (C≡N). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.75, 0.85 (6H, d, J = 6.8, CH_3), 0.92, 1.00, 1.15, 1.24, 1.37 (3H each, s, CH_3), 1.74 (3H, d, J = 6.6, H-33), 2.15–2.31 (2H, m, H-19, 20), 2.58, 2.69 (1H each, d, J = 18.0, H-1, AB system), 3.62 (3H, s, H-31), 5.03 (1H, q, J = 6.6, H-32). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.42, 14.66, 16.03, 19.13, 21.41, 21.83, 22.56, 22.68, 22.74, 22.92, 24.75, 26.99, 29.59, 29.71, 29.96, 31.80, 32.70, 37.17, 38.17, 40.51, 40.55, 42.68, 43.02, 44.11, 45.18, 46.36, 48.74, 51.13, 54.40, 57.01, 118.72 (C-2), 176.76 (C-28), 208.07 (C-3). Found, %: C, 66.88; H, 8.95; N, 2.47. $C_{33}H_{52}BrNO_3$. Calcd, %: C, 67.10; H, 8.87; N, 2.37.

General Method for Preparing 6 and 7. A solution of **3** or **4** (1 mmol) in *t*-BuOH (15 mL) was treated with *t*-BuOK (3 mmol). The reaction mixture was refluxed for 2 h, diluted with HCl solution (5%), and extracted with EtOAc (3 × 15 mL). The organic layer was washed with $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. The solvent was evaporated in a rotary evaporator. The solid was purified by CC with elution by petroleum ether–EtOAc (30:1).

Methyl 1-Cyano-3-ethyl-2-norlup-1(3)-en-28-oate (6). Yield 73%, R_f 0.52 (hexane–EtOAc, 10:1), colorless crystals, mp 223.9°C (hexane–EtOAc), $[\alpha]_D^{25}$ –6.7° (*c* 0.3, $CHCl_3$). IR (ν, cm^{-1}): 1594 (C=C), 1729 ($COOCH_3$), 2206 (C≡N). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.73, 0.84 (6H, d, J = 6.8, CH_3), 0.94, 0.95, 0.97, 1.04, 1.10 (3H each, s, CH_3), 1.13 (3H, t, J = 7.7, H-33), 2.15–2.24 (2H, m, H-19, 20), 2.28 (2H, q, J = 7.7, H-32), 3.64 (3H, s, H-31). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 13.72, 14.59, 14.78, 17.34, 17.37, 18.81, 20.49, 20.96, 22.25, 22.77, 22.87, 26.42, 27.21, 29.61, 29.74, 32.27, 35.08, 37.38, 38.09, 42.40, 43.04, 44.27, 46.98, 47.31, 48.99, 50.68, 51.09, 56.87, 62.52, 118.33 (C-2), 120.52 (C-1), 172.41 (C-3), 176.80 (C-28). Found, %: C, 80.34; H, 10.61; N, 2.75. $C_{33}H_{51}NO_2$. Calcd, %: C, 80.27; H, 10.41; N, 2.84.

2-Methyl-3-oxolup-1(2)-en-28-oic Acid Methyl Ester (7). Yield 43%, R_f 0.57 (hexane–EtOAc, 5:1), colorless crystals, mp 175.3°C (hexane–EtOAc), $[\alpha]_D^{22}$ +9.3° (*c* 0.5, $CHCl_3$). IR (ν, cm^{-1}): 1594 (C=C), 1667 (C=O), 1729 ($COOCH_3$). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.74, 0.86 (3H each, d, J = 6.8, CH_3), 0.95, 0.97, 1.00, 1.07, 1.08 (3H each, s, CH_3), 1.73 (3H, s, H-32), 2.15–2.31 (2H, m, H-19, 20), 3.64 (3H, s, H-31), 6.84 (1H, s, H-1). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 14.54, 14.65, 16.41, 16.92, 19.17, 19.37, 21.31, 21.54, 22.76, 22.93, 26.94, 28.35, 29.61, 29.73, 32.06, 33.94, 37.30, 38.39, 38.77, 41.56, 42.84, 44.19, 44.45, 44.70, 48.89, 51.14, 53.43, 57.00, 130.95 (C-2), 154.85 (C-1), 176.77 (C-28), 205.62 (C-3). Found, %: C, 79.44; H, 10.56. $C_{32}H_{50}O_3$. Calcd, %: C, 79.62; H, 10.44.

Screening for Cytotoxic Activity of 3–6. The cytotoxic activity of the synthesized compounds was determined by the classical MTT assay [19] using HEpG2, HCT116, MS, RD TE32, MCF-7, A549, and PC-3 cancer cell lines and HEK293 normal cells. A detailed description of the method has been published [11].

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