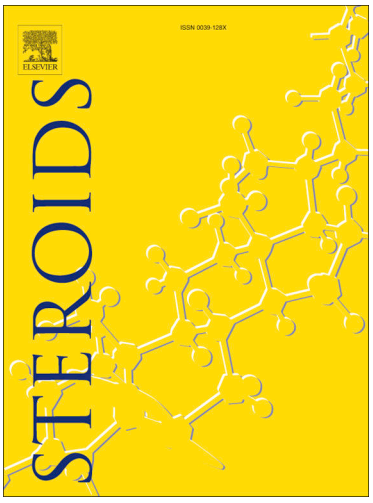


Synthesis and cytotoxicity of hybrids of 1,3,4- or 1,2,5-oxadiazoles tethered from ursane and lupane core with 1,2,3-triazole

Sergey A. Popov, Marya D. Semenova, Dmitry S. Baev, Tatiana S. Frolova, Michael A. Shestopalov, Chengzhang Wang, Zhiwen Qi, Elvira E. Shults, Māris Turks

PII: S0039-128X(20)30124-0
DOI: https://doi.org/10.1016/j.steroids.2020.108698
Reference: STE 108698

To appear in: *Steroids*

Received Date: 2 April 2020
Revised Date: 5 July 2020
Accepted Date: 11 July 2020

Please cite this article as: Popov, S.A., Semenova, M.D., Baev, D.S., Frolova, T.S., Shestopalov, M.A., Wang, C., Qi, Z., Shults, E.E., Turks, M., Synthesis and cytotoxicity of hybrids of 1,3,4- or 1,2,5-oxadiazoles tethered from ursane and lupane core with 1,2,3-triazole, *Steroids* (2020), doi: https://doi.org/10.1016/j.steroids.2020.108698

Synthesis and cytotoxicity of hybrids of 1,3,4- or 1,2,5-oxadiazoles tethered from ursane and lupane core with 1,2,3-triazole.

Sergey A. Popov[a*], Marya D. Semenova[a], Dmitry S. Baev[a] , Tatiana S. Frolova[b,c], Michael A. Shestopalov[d], Chengzhang Wang [e], Zhiwen Qi [e], Elvira E. Shults[a], Māris Turks[f].

[a]*Novosibirsk Institute of Organic Chemistry, Acad. Lavrentyev ave. 9, Novosibirsk, 630090, Russia*

[b]*The Federal Research Center Institute of Cytology and Genetics, Acad. Lavrentyev Ave., 10, 630090, Novosibirsk, Russia*

[c]*Novosibirsk State University, Pirogova Street, 2, 630090, Novosibirsk, Russia*

[d]*Nikolaev Institute of Inorganic Chemistry SB RAS, Acad. Lavrentiev ave., 3, 630090 Novosibirsk (Russia)*

[e]*Institute of Chemical Industry of Forest Products, Chinese academy of forestry, Nanjing 210042, China*

[f]*Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, P. Valdena Str. 3, Riga LV-1048, Latvia*

**Corresponding author. E-mail:spopov@nioch.nsc.ru*

Keywords: 1,3-cycloaddition; Triterpenoid conjugate; 1,2,3-Triazole; 1,3,4-, 1,2,5-Oxadiazole; Cytotoxicity tests; MDM-2-docking.

Abstract

Ursane and lupane type (1-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl and (1-((4-methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl hybrids were prepared by 1,3-cycloaddition reactions of azole-derived azides with alkyne esters connected to positions C-3 and C-28 of triterpene core and tested for cytotoxicity. Hybrid compounds of 1,3,4-oxadiazoles attached at positions 3- and 28- of triterpenoid frame *via* triazole spacer and combinations of 1,2,5-oxadiazole or 1,3,4-oxadiazole, tethered with succinate linker and 1,2,3-triazole at the position 3- of the ursane backbone, were inactive in relation to all the cancer cells tested. Eventually, combinations of furoxan fragment and 1,2,3-triazole linked to C-28 position of triterpene backbone demonstrated marked cytotoxic activity towards MCF-7 and HepG2 cells. The most active ester of ursolic acid with (1-((4-methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl substituent and 3-O-

acetyl group was superior in activity and selectivity over doxorubicin and ursolic acid on MCF-7 cells. The length of the carbon spacer group may be of crucial importance for cytotoxicity. The introduction of the additional ester linker between the C-28 of triterpenoid and triazole or changing triazole spacer between furoxan moiety and triterpenoid core resulted in activity decrease against all the tested cells. In accordance with molecular modeling results, the activity of new derivatives may be explained in terms of the interaction of the new hybrid molecules and Mdm2 binding sites.

1. Introduction

Pentacyclic triterpenoids available from the natural pool possess a wide range of biological activities that may be associated with the affinity of pentacyclic triterpenoids towards different biological targets [1-7]. Ursane and lupane natural triterpenoids generally exhibit only moderate anti-inflammatory and anti-proliferative activity and are regarded as promising scaffolds for the synthesis of new derivatives with pronounced anti-inflammatory properties or selective cytotoxic effects against series of different cancer cells [8-10].

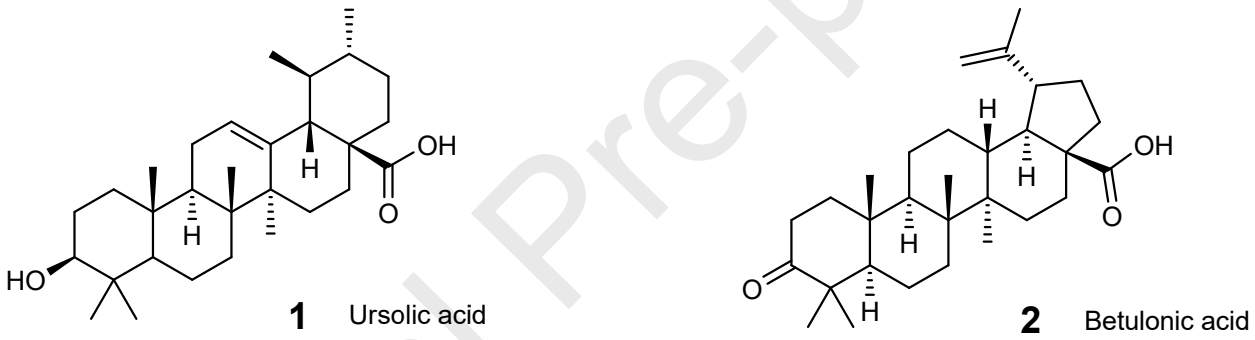


Fig.1 Structures of ursolic and betulonic acids.

Accessible and non-toxic ursolic and semisynthetic betulonic acids (Fig. 1) are widely used as starting material for the preparation of novel bioactive derivatives. A synthetic combination of various pharmacophores with triterpenoid molecules can lead to hybrid compounds with improved physicochemical properties and / or new types of biological activity [11-13].

The addition of certain of heterocyclic moieties, including azoles (1,3,4-oxadiazoles, 1,2,5-oxadiazoles, and 1,2,3-triazoles) to the triterpenoid framework opens routes to compounds exhibiting more pronounced antiproliferative or chemopreventive activity that may be due to enhanced binding to a series of corresponding molecular targets or bioavailability improvement. [14-16, 19-25]

Due to the availability of alkyne-derived esters and amides of triterpenoids [14-16] and development of methods of selective Cu-catalyzed 1,3 –cycloaddition [17,18] the syntheses of lupane and ursane conjugates including 1,2,3- triazole functionalities are among the most researched. Lupane and ursane moieties were connected using 1,3-dipolar cycloaddition to afford hybrids with triazole linker that

exerted excellent activity against breast cancer cells [14]. Ursolic acid hybrids with AZT bearing 1,2,3- triazole linkage attached via C-28 amide or ester with moderate cytotoxic activity were obtained [19,20]. Ursonic acid-derived compounds comprising aryl-1,2,3- triazole pendant groups at the C-28 position of triterpenoid frame demonstrated superior activity towards different malignant cells [21]. Similarly, triazole-bearing lupane-type esters with C-3 keto group, [15] and (1-aryl-1H-1,2,3-triazol-4-yl)methyl oleanonic esters [22,23] might display better cytotoxic activity comparing corresponding triterpenic acid hybrids with 3-OH group. 1,2,3-Triazole lupane conjugates with peptides were synthesized through 1,3-dipolar cycloaddition of azido peptides to (3-oxolup-20(29)-en-28-oyl)-4-ethynyl aniline [24]. The synthetic amino acid conjugates with 1,2,3-triazole and betulonic acid of other type exerted good anti-inflammatory activity[25]. In this regard, the preparation of new C-3-keto hybrids including 1,2,3-triazole group starting from the available betulonic acid is of interest for the further study of anticancer activity. Combined derivatives of other oxadiazole, 1,2,5-oxadiazole-2-oxide (furoxan) displayed activity as NO donors [26] and preparation and cytotoxicity of furoxan hybrids with triterpenoids have been studied [27,28].

A series of derivatives with furoxan-type substituents and different linker groups attached to the positions C-28 and C-3 of lupane and ursane backbones were characterized by significant activity against cancer cells [27,28]. Thus, furoxan derivatives spacered from ursane core by the 3-O-succinate group were prepared, and some of them were found potently cytotoxic [27] against HepG2 cells. The results of work [28] showed that furoxan-holding betulonic acid hybrids were superior in antitumor activity against HepG2 and B16 cell lines over nitrate-based lupane conjugates. Betulonic acid C-28 hybrids were found more cytotoxic than betulinic acid-furoxan derivatives attached at C-3 *via* succinate linker. Fewer works focused conjugates of ursane and lupane derivatives with 1,3,4 oxadiazoles. The synthesis and the moderate cytotoxicity of lupane-type hybrids with 1,3,4-oxadiazoles were reported [29]. Ursane derived esters with aryl oxadiazoles substituents were found non-cytotoxic but possessing promising anti-inflammatory action [30]. Recently, we reported the preparation of low toxic conjugates with anti-inflammatory properties including 1,2,5- and 1,3,4-oxadiazoles linked to betulonic acid core via aminoacid functionalities [31]. Certain azoles are bioisosteres of amides and peptides, therefore, the introduction of a sequence of different heterocycles at the triterpene backbone may lead to hybrids with improved target affinity for cytotoxicity or anti-inflammatory activity. In the literature, there is no information on hybrids of 1,3,4- or 1,2,5-oxadiazoles connected to different positions of triterpenoids through 1,2,3- triazole linker. We were interested in synthetic combination of two different types of azoles (1,3,4- oxadiazole and 1,2,3-triazole or 1,2,5- oxadiazole and 1,2,3- triazole) as pendant groups at different positions of the triterpenoid frame for the subsequent study of cytotoxic activity of novel compounds. Thus, the objective of the present work was to obtain novel ursane and lupane conjugates using 1,3-

cycloaddition of azides - derived from 1,3,4- and 1,2,5- oxadiazoles to propargyl esters of triterpenoids, and to study the cytotoxicity of the hybrid compounds. To estimate affinity to molecular targets, docking of the novel triterpenoid hybrid derivatives in Mdm2 binding sites was performed and supported with flow cytometry data.

2. Experimental

General

IR spectra were recorded on a Bruker Vector 22 FTIR spectrometer in KBr pellets. The mass spectra were recorded on a Thermo Scientific DFS high-resolution mass spectrometer (evaporator temperature 200–250°C, EI ionization at 70 eV). ^{1}H and ^{13}C NMR spectra were registered on a Bruker AV-300 (300.13 MHz for ^{1}H, 75.48 MHz for ^{13}C), Bruker AV-400 (400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C) and DRX-500 (operating frequencies 500.13 and 125.76 MHz, respectively) at room temperature. The chemical shifts are given in ppm relative to signals of the solvents used as internal standards: in ^{1}H NMR spectra δ_H 7.24 ($CHCl_3$) and ^{13}C NMR spectra δ_C 76.90 ($CDCl_3$). Signals in the NMR ^{1}H and ^{13}C spectra of ursane series were assigned by correlation with those of ursolic acid [32] and ursolic acid acetate [33]. Signals in the NMR ^{1}H and ^{13}C spectra of lupane series were assigned by correlation with those of betulonic acid (in $CDCl_3$) [34] *J* values are given in Hertz. The reaction progress was controlled by TLC on Sorbfil UV-254 plates using MTBE and 1:1 MTBE–hexane mixture as eluents, with visualization by $FeCl_3$ solution and under UV light. The reaction products were isolated by column chromatography using silica gel from Acros (35-70 μm). In the NMR ^{1}H and ^{13}C spectra the assignments marked with the same symbols $^{\S, \#}$ are interchangeable.

2.1.1. Methods and reagents

Propargyl betulinate **13b** [35], 2-ethoxy-2-oxoethyl ursolate **5** [36] were prepared as reported before. 2-(Chloromethyl)-5-aryl-1,3,4-oxadiazoles **3a-d** [37], furoxane derivative 3-(bromomethyl)-4-methyl-1,2,5-oxadiazole-2-oxide **4** [38], propargyl 4-chloro-4-oxobutanoate [39], (4-methyl-1,2,5-oxadiazole-2-oxide-3-yl)methyl-N-(3-oxo-lup-20 (29)-en-28-oyl)-2-aminoethanoate **16a**, (4-methyl-1,2,5-oxadiazole-2-oxide-3-yl)methyl-N-(3-oxo-lup-20 (29)-en-28-oyl)-3-aminopropanoate **16b**, (4-methyl-1,2,5-oxadiazole-2-oxide-3-yl)methyl-N-(3-oxo-lup-20 (29)-en-28-oyl)-4-aminobutyrate **16c** [31] were synthesized following corresponding previously published procedures.

2.1.2. Synthesis of intermediate and hybrid compounds

General procedure for the preparation of 2-aziadomethyl-5-aryl-1,3,4-oxadiazoles 3'a, b, c, d, and 3-(azidomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide 4 '.

2-(Chloromethyl) -5-aryl-1,3,4-oxadiazole **3a, b, c, d** (1 mmol) or 3-(bromomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide **4** and sodium azide (3 mmol) in DMF (10 mL) was stirred at 50-55° C for 5-7 hours, (TLC control). The reaction mixture was poured into ice water, extracted with MTBE (3 × 30 mL). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuum. The product was purified by chromatography over a short silica gel column (CH_2Cl_2-MTBE) to afford azides **3'a, b, c,d** or **4'.**

3-(Azidomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide 4'

The title compound was prepared from **4** in 98% yield as pale yellow oil.

IR (cm^{-1}): 500, 554, 594, 658, 704, 773, 849, 883, 941, 993, 1039, 1093, 1122, 1221, 1271, 1315, 1352, 1387, 1427, 1477, 1520, 1606, 1674, 2116, 2935. NMR ^1H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 2.35 (3H, s, Me-1), 4.31 (2H, s, 2H-4), NMR ^{13}C (125.76 MHz , $CDCl_3$**,** δ, ppm, J/Hz**)**: 10.8 (C-1), 42.3 (C-4), 112.3 (C-2), 153.9 (C-3). Found, m/z: 155,0448 [M]+ . $C_4H_5N_5O_2$ Calculated, m/z: 155,0443.

Synthesis of propargyl ursolate 6a and propargylbetulonate 2a.

A suspension of ursolic acid 1 or betulonic acid 2 (10 mmol), K_2CO_3 (1 mol), KI (0.1 mmol) in acetone (50 mL) and propargyl bromide (12 mmol) was stirred for 20-24 hours at room temperature (TLC control). The solvent was removed in vacuo, the residue was suspended in water. The precipitate was separated on a filter, washed with HCl aq. (3%, 30 mL), water (2×50 mL), and air-dried to give **2a** (82%), **6a** (84%). ^1H, ^{13}C NMR spectra of compounds **2a**, **6a** were identical to those published [35].

3-(prop-1-yn)oxy-2-oxoethyl ursolate ***(6b)***

A suspension of ursolic acid carboxymethyl ester **5b** (1 mmol), K_2CO_3 (10 mmol), KI (0.1 mmol) in DMF (15 mL), propargyl bromide (1.1 mol) was stirred at room temperature for 20-24 hours (TLC control). The reaction mixture was poured into water, acidified with 10% HCl to pH ~ 1. The precipitate was separated by filtration, rinsed with water (3×20 mL), air-dried, chromatographed over a short silica gel column (CH_2Cl_2-MTBE), to afford ester **6b** as off-white powder, 98%.

IR (KBr, cm^{-1}): 434, 451, 527, 602, 631, 677, 708, 756, 791, 810, 833, 866, 935, 970, 997, 1028, 1051, 1078, 1115, 1140, 1165, 1192, 1227, 1252, 1273, 1308, 1329, 1360, 1383, 1419, 1448, 1464, 1657, 1726, 1772, 2135, 2856, 2926, 2941, 2968, 3292, 3437, 3537. NMR ^1H (400.13 MHz , $CDCl_3$**,** δ, ppm, J/Hz**):** 0.69 (3H, s, Me-26), 0.74 (3H, s, Me-25), 0.82 (3H, d, $J_{29\text{-}19}$ = 5.9 Hz, Me-29), 0.87 (3H, Me-24), 0.91 (3H, d, $J_{30\text{-}18}$ = 5.1 Hz, Me-30), 0.94 (3H, s, Me-23), 1.04, (3H, s, Me-27), 2.21 (1H, d, $J_{18\text{-}19}$

= 11.1 Hz, H-18), 2.46 (1H, s, H-3'), 3.12-3.21 (1H, m, H-3), 4.54 (2H, AB, J_{AB} = 16.1 Hz, Δη = 22.01, 2H-3'), 4.69 (2H, s, 2H-1'), 5.20 (1H, br.s, H-12). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm, J/Hz): 15.4 (C-25, q), 15.6 (C-24, q), 16.9 (C-26, q), 17.0 (C-29, q), 18.2 (C-6, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-27, t), 24.1 (C-16, q), 27.2 (C-2, t), 27.9 (C-15, t), 28.1 (C-23, q), 30.6 (C-21, t), 32.9 (C-7, t), 36.4 (C-22, t), 36.9 (C-10, s), 38.6 (C-1, t), 38.7 (C-4, s), 38.7 (C-20, d), 39.0 (C-19, d), 39.5 (C-8, s), 42.0 (C-14, s), 47.5 (C-9, d), 48.1 (C-17, s), 52.5 (C-3', t), 52.6 (C-18, d), 55.1 (C-5, d), 60.1 (C-1', t), 75.5 (C-5', d), 76.9 (C-4', c), 78.9 (C-3, d), 125.7 (C-12, d), 137.8 (C-13, s), 167.3 (C-2', s), 176.7 (C-28, s). Found, m/z: 552.3809 [M]+ . $C_{35}H_{52}O_5$ Calculated, m/z: 552.3812

3β-(4-Oxo-4-(prop-2-ynyloxy)-butanoyloxy)-urs-12-en-28-oic acid ***(10)***

To a stirred mixture of ursolic acid **1** (0.1 mmol), 4-DMAP (0.01 mmol) in $CHCl_3$ (25 mL) and pyridine (8 mL) a solution of propargyl 4-chloro-4-oxobutanoate (0.4 mmol) in $CHCl_3$ (20 mL) was added dropwise at 0- 5ºC. The reaction mixture was stirred at room temperature for 48 h.

The solvent was distilled off in vacuo, $H_2SO_{4\ aq}$, (5%, 50 mL) was added dropwise to the residue. The mixture was extracted with MTBE (3 × 30 mL). The organic phase was washed with water (2 × 30 ml), dried (Na_2SO_4), concentrated in vacuo, chromatographed (SiO_2, CCl_4-MTBE), and concentrated to afford succinate **10**, 87%.

IR (KBr, cm^{-1}): 544, 571, 663, 771, 806, 829, 860, 883, 968, 993, 1026, 1065, 1101, 1157, 1211, 1240, 1257, 1273, 1315, 1350, 1375, 1414, 1458, 1689, 1736, 2131, 2623, 2874, 2929, 2941, 2976, 3292. NMR ^1H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.73 (3H, c, Me-26), 0.82 (6H, s, Me-24, Me-25), 0.84 (3H, s Me-29), 0.93 (6H, c, Me-23, Me-30), 1.04 (3H, s, Me-27), 2.15 (1H, d $J_{18\text{-}19}$ = 10.9 Hz, H-18), 2.45 (1H, s, H-7'), 2.59-2.70 (4H, m, 2H-2', 2H-3'), 4.46-4.53 (1H, m, H-3), 4.67 (2H, ddd, J^1=J^2 = 2.6 Hz, 2H-5'), 5.18-5.22 (1H, m, H-12). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.4 (C-25), 16.6 (C-24), 16.8 (C-26), 16.9 (C-29), 18.0 (C-6), 21.1 (C-30), 23.1 (C-11), 23.4 (C-2), 23.4 (C-27), 24.0 (C-16), 27.9 (C-15), 27.9 (C-23), 29.0 (C-3'), 29.4 (C-2'), 30.5 (C-21), 32.8 (C-7), 36.7 (C-22), 36.7 (C-10), 37.6 (C-1), 38.1 (C-4), 38.7 (C-20), 38.9 (C-19), 39.3 (C-8), 41.9 (C-14), 47.3 (C-9), 47.7 (C-17), 52.1 (C-5'), 52.6 (C-18), 55.2 (C-5), 75.0 (C-7'), 77.5 (C-6'), 81.5 (C-3), 125.3 (C-12), 138.0 (C-13), 171.6 (C-4'), 171.9 (C-1'), 181.4 (C-28). Found, m/z: 594.3923 [M]+ . $C_{37}H_{54}O_6$ Calculated, m/z: 594.3915

Synthesis of triterpenoid 5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl esters 7, 14; 4-methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl esters 8, 13 by the interaction of 2- (azidomethyl) -5-methyl-1,3,4-oxadiazoles 3'or 3- (azidomethyl) -4-methyl-1,2,5-oxadiazole 2-oxide 4 'with propargyl ethers 6a, 6b, 2a

A mixture of triterpenoid derived propargyl ester **6a, 6b, 2a** (1 mmol), 2-azidomethyl-5-aryl-1,3,4-oxadiazol **3'** or 3-(azidomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide **4'** (1 mmol), $CuSO_4 \times 5H_2O$ (0,2 mmol) in DMF (15 mL) was stirred at ambient temperature for 30 min, followed by Na ascorbate (0,2 mmol) addition. The mixture was stirred at 50ºC for 10-15 h. (TLC control). The reaction mixture was poured into ice water, acidified with 10% H_2SO_4 solution to pH ~3, the precipitate was filtered off, washed with water to pH ~ 7, and dried on air. The product was purified by column chromatography (SiO_2, CCl_4-MTBE).

(((5-Phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl-3β -hydroxy-urs-12-en-28-oate ***(7a)***

The title compound was prepared in the reaction of ester **6a** with azide **3'a** as white powder in 65% yield. IR (KBr, cm^{-1}): 498, 517, 536, 559, 604, 663, 690, 712, 733, 756, 806, 829, 866, 912, 951, 964, 974, 995, 1030, 1045, 1072, 1092, 1107, 1140, 1167, 1180, 1198, 1228, 1269, 1284, 1306, 1360, 1379, 1387, 1450, 1483, 1552, 1578, 1591, 1610, 1657, 1722, 2247, 2872, 2926, 2945, 3149, 3435. NMR ^1H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.53 (3H, s, Me-26), 0.73 (3H, s, Me-25), 0.79 (3H, d, $J_{29\text{-}19}$ = 6.8 Hz, Me-29), 0.83 (3H, s, Me-24), 0.89 (3H, br.s., Me-30), 0.94 (3H, s, Me-23), 1.02 (3H, s, Me-27), 2.17 (1H, d, $J_{18\text{-}19}$ = 11.4 Hz, H-18), 3.14-3.20 (1H, m, H-3), 5.08-5.20 (3H, m (d + AB), H-12, 2H-1'), 5.83 (2H, AB-sys, J_{AB} = 16.2, Δη = 6.9, H-4'), 7.45-7.58 (3H, m, H-9', H-10', H-11'), 7.78 (1H, s, H-3'), 7.97-8.03 (2H, m, H-8', H-12'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.4 (C-25, q), 15.6 (C-24, q), 16.7 (C-26, q), 16.9 (C-29, q), 18.2 (C-6, t), 21.0 (C-30, q), 23.2 (C-11, t), 23.4 (C-27, q), 24.1 (C-16, t), 27.1 (C-2, t), 27.9 (C-15, t), 28.1 (C-23, q), 30.5 (C-21, t), 32.9 (C-7, t), 36.5 (C-22, t), 36.8 (C-10, s), 38.5 (C-1, t), 38.7 (C-4, s), 38.7 (C-20, d), 39.0 (C-19, d), 39.4 (C-8, s), 42.0 (C-14, s), 44.1 (C-4', t), 47.4 (C-9, d), 48.1 (C-17, s), 52.7 (C-18, d), 55.1 (C-5, d), 57.2 (C-1', t), 78.9 (C-3, d), 122.9 (C-7', s), 124.3 (C-3', d), 125.6 (C-12, d), 127.1 (C-8', d), 127.1 (C-12', d), 129.1 (C-9', d), 129.1 (C-11', d), 132.3 (C-10', d), 137.9 (C-13, s), 144.2 (C-2', s), 159.8 (C-5', s), 166.1 (C-6', s), 177.3 (C-28, s). Found, m/z: 695.4404 [M]+ . $C_{42}H_{57}O_4N_5$ Calculated, m/z: 695.4405

(((5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl-3β -hydroxy-urs-12-en-28-oate ***(7c)***

The title compound was prepared in the reaction of ester **6a** with azide **3'c** as white powder in 55% yield. IR (KBr, cm^{-1}): 461, 478, 505, 536, 559, 627, 660, 677, 694, 731, 791, 804, 833, 951, 976, 997, 1011, 1030, 1045, 1084, 1109, 1140, 1169, 1178, 1200, 1,2,3-0, 1269, 1306, 1325, 1365, 1379, 1387, 1408, 1458, 1481, 1547, 1585, 1605, 1655, 1724, 2872, 2926, 2970, 3090, 3149, 3437. NMR ^1H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.53 (3H, s, Me-26), 0.74 (3H, s, Me-25), 0.80 (3H, d, $J_{29\text{-}19}$ = 6.3 Hz, Me-29), 0.84 (3H, s, Me-24), 0.89 (3H, br.s, Me-30), 0.95 (3H, s, Me-23), 1.02 (3H, s, Me-27),

2.17 (1H, d $J_{18\text{-}19}$ = 10.8 Hz, H-18), 3.12-3.23 (1H, m, H-3), 5.08-5.21 (3H, m, H-12, 2H-1'), 5.30 (2H, m, H-4'), 7.64 (2H, d, J = 8.2 Hz, H-9', H-11'), 7.77 (1H, s, H-3'), 7.87 (2H, d, J = 8.2 Hz, H-8', H-12'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.4 (C-25, q), 15.6 (C-24, q), 16.7 (C-26, q), 16.9 (C-29, q), 18.2 (C-8, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-27, q), 24.1 (C-16, t), 27.1 (C-2, t), 27.9 (C-15, t), 28.1 (C-23, q), 30.5 (C-21, t), 32.9 (C-7, t), 36.5 (C-22, t), 36.8 (C-10, s), 38.5 (C-1, t), 38.6 (C-4, s), 38.7 (C-20, d), 39.0 (C-19, d), 39.4 (C-8, s), 41.9 (C-14, s), 44.1 (C-4', t), 47.4 (C-9, d), 48.0 (C-17, s), 52.7 (C-18, d), 55.1 (C-5, d), 57.1 (C-1', t), 78.9 (C-3, d), 121.7 (C-10', s), 124.4 (C-3', d), 125.6 (C-12, d), 127.3 (C-7', s), 128.4 (C-8', d), 128.4 (C-12', d), 132.5 (C-9', d), 132.5 (C-11', d), 137.8 (C-13, s), 144.3 (C-2', s), 160.0 (C-5', s), 165.5 (C-6', s), 177.4 (C-28, s). Found, m/z: 773.3505 [M]+ $C_{42}H_{56}O_4N_5{}^{79}Br_1$. Calculated, m/z: 773.3510.

(((5-(3,4-Dichlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl-3β -hydroxy-urs-12-en-28-oate ***(7d)***

The title compound was prepared in the reaction of ester **6a** with azide **3'd** as white powder in 37% yield. IR (KBr, cm^{-1}): 440, 461, 501, 555, 604, 660, 677, 729, 766, 812, 829, 849, 889, 912, 951, 976, 995, 1034, 1045, 1086, 1105, 1138, 1169, 1198, 1,2,3-2, 1269, 1306, 1325, 1365, 1387, 1408, 1456, 1543, 1566, 1581, 1606, 1724, 2872, 2926, 2945, 2970, 3091, 3147, 3439. NMR ^1H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.53 (3H, s, Me-26), 0.73 (3H, s, Me-25), 0.80 (3H, d, $J_{29\text{-}19}$ = 6.5 Hz, Me-29), 0.84 (3H, s, Me-24), 0.89 (3H, br.s, Me-30), 0.95 (3H, s, Me-23), 1.02 (3H, s, Me-27), 2.17 (1H, d $J_{18\text{-}19}$ = 11.5 Hz, H-18), 3.14-3.22 (1H, m, H-3), 5.09-5.21 (3H, m, H-12, 2H-1'), 5.83 (2H, AB-sys, J_{AB} = 16.3, Δη = 6.2, 2H-4'), 7.58 (1H, d, J = 8.4, H-9'), 7.78 (1H, s, H-3'), 7.85 (1H, dd, J_1 = 8.6, J_2 = 2.0 Hz, H-8'), 8.10 (1H, d, J = 2.0 Hz, H-12'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.4 (C-25, q), 15.6 (C-24, q), 16.6 (C-26, q), 16.9 (C-29, q), 18.1 (C-8, t), 21.0 (C-30, q), 23.1 (C-11, t), 23.3 (C-27, q), 24.0 (C-16, t), 27.1 (C-2, t), 27.8 (C-15, t), 28.0 (C-23, q), 30.4 (C-21, t), 32.8 (C-7, t), 36.4 (C-22, t), 36.8 (C-10, s), 38.5 (C-1, t), 38.6 (C-4, s), 38.6 (C-20, d), 38.9 (C-19, d), 39.3 (C-8, s), 41.9 (C-14, s), 44.0 (C-4', t), 47.3 (C-9, d), 48.0 (C-17, s), 52.7 (C-18, d), 55.0 (C-5, d), 57.1 (C-1', t), 78.8 (C-3, d), 122.5 (C-7', s), 124.5 (C-3', d), 125.5 (C-12, d), 126.0 (C-8', d), 128.7 (C-12', d), 131.3 (C-9', d), 133.8 (C-10', s), 137.0 (C-11', s), 137.8 (C-13, s), 144.2 (C-2', s), 160.3 (C-5', s), 164.3 (C-6', s), 177.3 (C-28, s). Found, m/z: 763.3616 [M]+ $C_{42}H_{55}O_4N_5{}^{35}Cl_2$ Calculated, m/z: 763.3626.

(1-((4-Methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl-3β-hydroxy-urs-12-en-28-oate ***(8a)***

The title compound was prepared in the reaction of ester **6a** with azide **4'** as white powder in 67% yield. IR (KBr, cm^{-1}): 500, 517, 559, 600, 661, 679, 700, 725, 795, 829, 849, 914, 951, 976, 995, 1043, 1080, 1107, 1140, 1169, 1198, 1228, 1269, 1308, 1327, 1387, 1456, 1520, 1552, 1564, 1610, 1657,

1722, 2872, 2928, 2945, 2964, 3147, 3457. NMR ^{1}H (400.13 MHz , $CDCl_3$ δ, ppm, J/Hz): 0.53 (3H, s, Me-26), 0.75 (3H, s, Me-25), 0.81 (3H, d, $J_{29\text{-}19}$ = 6.6 Hz, Me-29), 0.87 (3H, s, Me-24), 0.88-0.92 (3H, br.s., Me-30), 0.96 (3H, s, Me-23), 1.04 (3H, s, Me-27), 2.18 (1H, d, $J_{18\text{-}19}$ = 11.4 Hz, H-18), 2.42 (3H, s, Me-7'), 3.15-3.21 (1H, m, H-3), 5.11 (2H, AB, J_{AB} = 12.9, Δη = 13.3, 2H-1'), 5.20 (1H, ddd, J_1 = 3.5, J_2 = 3.8, H-12), 5.40 (2H, s, 2H-4'). 7.71 (1H, s, H-3'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 10.9 (C-7', q), 15.4 (C-25, q), 15.6 (C-24, q), 16.7 (C-26, q), 16.9 (C-29, q), 18.3 (C-6, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-27, q), 24.2 (C-16, t), 27.2 (C-2, t), 27.9 (C-15, t), 28.1 (C-23, q), 30.6 (C-21, t), 33.0 (C-7, t), 36.5 (C-22, t), 36.9 (C-10, s), 38.6 (C-1, t), 38.7 (C-4, s), 38.8 (C-20, d), 39.0 (C-19, d), 39.5 (C-8, s), 41.2 (C-4', t), 42.0 (C-14, s), 47.5 (C-9, d), 48.1 (C-17, s), 52.8 (C-18, d), 55.2 (C-5, d), 57.1 (C-1', t), 78.9 (C-3, d), 111.8 (C-6', s), 124.5 (C-3', d), 125.7 (C-12, d), 137.9 (C-13, s), 144.2 (C-2', s), 153.9 (C-5', s), 177.3 (C-28, s). Found, m/z: 649.4186 [M]+ $C_{37}H_{55}O_5N_5$ Calculated, m/z: 649.4198

3-((4-((Ursoloyloxyacetoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-methyl-1,2,5-oxadiazole 2-oxide ***(9a)***

The title compound was prepared in the reaction of ester **6b** with azide **4'** as white powder in 84% yield. IR (KBr, cm^{-1}): 500, 563, 602, 661, 700, 723, 756, 791, 829, 849, 914, 951, 976, 995, 1045, 1080, 1113, 1138, 1167, 1180, 1194, 1230, 1275, 1306, 1327, 1377, 1387, 1423, 1456, 1520, 1562, 1610, 1740, 1765, 2872, 2928, 2945, 2968, 3145, 3444.NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.69 (3H, s, Me-26), 0.76 (3H, s, Me-25), 0.83 (3H, d, $J_{29\text{-}19}$ = 6.3 Hz, Me-29), 0.89 (3H, s, Me-24), 0.92 (3H, br.s, Me-30), 0.96 (3H, s, Me-23), 1.06 (3H, s, Me-27), 2.21 (1H, d $J_{18\text{-}19}$ = 11.2 Hz, H-18), 2.42 (3H, s, Me-9'), 3.15-3.22 (1H, m, H-3), 4.51 (2H, AB-sys, J_{AB} = 15.9, Δη = 24.8, 2H-1'), 5.22 (1H, ddd, J_1 = J_2 = 3.8, H-12), 5.25 (2H, s, 2H-3'), 5.42 (2H, s, 2H-6'), 7.77 (1H, s, H-5'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 10.9 (C-9', q), 15.2 (C-25, q), 15.6 (C-24, q), 16.9 (C-26, q), 16.9 (C-29, q), 18.3 (C-6, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-27, q), 24.1 (C-16, t), 27.2 (C-2, t), 27.9 (C-15, t), 28.1 (C-23, q), 30.6 (C-21, t), 33.0 (C-7, t), 36.4 (C-22, t), 36.9 (C-10, s), 38.6 (C-1, t), 38.7 (C-4, s), 38.7 (C-20, d), 39.0 (C-19, d), 39.5 (C-8, s), 41.3 (C-6', t), 42.0 (C-14, s), 47.5 (C-9, d), 48.1 (C-17, s), 52.7 (C-18, d), 55.2 (C-5, d), 57.8 (C-3', t), 60.2 (C-1', t), 78.9 (C-3, d), 111.8 (C-8', s), 124.5 (C-5', d), 125.7 (C-12, d), 137.8 (C-13, s), 143.3 (C-4', s), 153.9 (C-7', s), 167.8 (C-2', s), 176.8 (C-28, s). Found, m/z: 707.4262 [M]+ $C_{39}H_{57}O_7N_5$ Calculated, m/z: 707.4253

Preparation of 3-O-succinyl-oxadiazoles 11 a-d, 12.

A solution of ursolic acid (4-propargyl)-succinate **10** (0,1 mmol), 2-(azidomethyl)-5-aryl-1,3,4-oxadiazole **3'a-d** or 3-(azidomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide **4'** (0,1 mmol) and

diisopropylethylamine (1 mmol) in DMF (20 mL) was stirred at ambient temperature for 30 min, followed by addition of CuBr (0.01 mmol). The mixture was stirred under N_2 at 50-55ºC for 48 h (TLC control). The reaction mixture was poured onto ice and acidified to pH~3 with $H_2SO_{4\ aq}$ (10%) The precipitate was filtered, washed with water to pH ~7, and dried on air. The products were purified by column chromatography (SiO_2, MTBE -CCl_4).

3 β-(4-Oxo-4-((1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-methoxy)-butanoyloxy)-urs-12-en-28-oic acid ***(11a).***

The title compound was prepared in the reaction of propargyl ester **10** with azide **3'a** as white powder in 37% yield. IR (KBr, cm^{-1}): 569, 661, 690, 712, 744, 775, 806, 829, 858, 881, 908, 928, 966, 993, 1016, 1047, 1070, 1090, 1105, 1161, 1,2,3-4, 1254, 1271, 1315, 1369, 1377, 1389, 1421, 1452, 1483, 1552, 1578, 1591, 1610, 1693, 1732, 2623, 2874, 2928, 2945, 2970, 3145, 3442. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.74 (3H, s, Me-26), 0.79 (3H, s, Me-25), 0.81 (3H, s, Me-24), 0.83 (3H, d, $J_{29\text{-}19}$ = 6.4 Hz, Me-29), 0.91 (3H, s, Me-23), 0.93 (3H, s, Me-30), 1.05 (3H, s, Me-27), 2.16 (1H, d $J_{18\text{-}19}$ = 11.0 Hz, H-18), 2.61 (4H, m, 2H-2', 2H-3'), 4.43-4.50 (1H, m, H-3), 5.18-5.22 (1H, m, H-12), 5.22-5.26 (2H, m, 2H-5'), 5.85 (2H, c, 2H-8'), 7.45-7.57 (3H, m, H-13', H-14', H-15'), 7.84 (1, s, H-7'), 8.00 (2H, d, J = 7.2 Hz, H-12', H-16'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.4 (C-25, q), 16.6 (C-24, q), 16.9 (C-26, q), 17.0 (C-29, q), 18.0 (C-6, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-2, t), 23.5 (C-27, q), 24.0 (C-16, t), 27.9 (C-15, t), 28.0 (C-23, q), 29.0 (C-2', t), 29.3 (C-3', t), 30.5 (C-21, t), 32.7 (C-7, t), 36.6 (C-22, t), 36.8 (C-10, s), 37.6 (C-1, t), 38.1 (C-4, s), 38.7 (C-20, d), 38.9 (C-19, d), 39.4 (C-8, s), 41.8 (C-14, s), 44.1 (C-8', t), 47.3 (C-9, d), 47.8 (C-17, s), 52.4 (C-18, d), 55.2 (C-5, d), 57.6 (C-5', t), 81.3 (C-3, d), 122.9 (C-11', s), 124.2 (C-7', d), 1,2,5-.5 (C-12, d), 127.1 (C-12', d), 127.1 (C-16', d), 129.1 (C-13', d), 129.1 (C-15', d), 132.3 (C-14', d), 137.9 (C-13, s), 143.8 (C-6', s), 159.9 (C-9', s), 166.1 (C-10', s), 171.7 (C-4', s), 172.1 (C-1', s), 183.6 (C-28, s). Found, m/z: 795.4559 [M]+ $C_{46}H_{61}O_7N_5$ Calculated, m/z: 795.4566

3β-(4-Oxo-4-((1-((5-(3,5-dichlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-methoxy)-butanoyloxy)-urs-12-en-28-oic acid ***(11b)***

The title compound was prepared in the reaction of propargyl ester **10** with azide **3'b** as white powder in 27% yield. IR (KBr, cm^{-1}): 519, 548, 571, 667, 729, 771, 806, 866, 908, 951, 968, 987, 1030, 1049, 1105, 1161, 1238, 1255, 1271, 1315, 1377, 1390, 1412, 1446, 1460, 1514, 1547, 1570, 1691, 1732, 2621, 2874, 2928, 2972, 3078, 3145, 3439. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.76 (3H, s, Me-26), 0.80 (6H, s, Me-25, Me-24), 0.84 (3H, d, $J_{29\text{-}19}$ = 6.4 Hz, Me-29), 0.91 (3H, s, Me-23), 0.93 (3H, s, Me-30), 1.06 (3H, s, Me-27), 2.16 (1H, d $J_{18\text{-}19}$ = 11.7 Hz, H-18), 2.57-2.66 (4H, m, 2H-2', 2H-3'), 4.43-4.50 (1H, m, H-3), 5.21-5.27 (3H, m, H-12, 2H-5'), 5.86 (2H, c, 2H-8'), 7.53 (1H, br.s, H-

14'), 7.83 (1H, s, H-7'), 8.00 (2H, s, H-12', H-16'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm, Hz): 15.4 (C-25, q), 16.7 (C-24, q), 17.0 (C-26, q), 17.0 (C-29, q), 18.0 (C-6, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-2, t), 23.5 (C-27, q), 24.0 (C-16, t), 27.9 (C-15, t), 28.0 (C-23, q), 29.0 (C-2', t), 29.3 (C-3', t), 30.5 (C-21, t), 32.7 (C-7, t), 36.7 (C-22, t), 36.8 (C-10, s), 37.6 (C-1, t), 38.1 (C-4, s), 38.7 (C-20, d), 38.9 (C-19, d), 39.4 (C-8, s), 41.8 (C-14, s), 44.1 (C-8', t), 47.3 (C-9, d), 47.8 (C-17, s), 52.4 (C-18, d), 55.1 (C-5, d), 57.6 (C-5', t), 81.4 (C-3, d), 124.4 (C-7', d), 125.3 (C-12', d), 1,2,5-.3 (C-16', d), 125.4 (C-11', s), 1,2,5-.5 (C-12, d), 132.2 (C-14', d), 136.1 (C-13', s), 136.1 (C-15', s), 137.9 (C-13, s), 143.9 (C-6', s), 160.5 (C-9', s), 164.0 (C-10', s), 171.8 (C-4', s), 172.1 (C-1', s), 183.4 (C-28, s). Elemental analysis: found C 63.81%, H 6.83%, Cl 8.20% N 8.15%; calculated C 63.88%, H 6.88%, Cl 8.20%, N 8.10%.

3β-(4-Oxo-4-((1-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-butanoyloxy)-urs-12-en-28-oic acid ***(11c)***

The title compound was prepared in the reaction of propargyl ester **10** with azide **3'c** as white powder in 33% yield. IR (KBr, cm^{-1}): 461, 503, 571, 661, 679, 694, 731, 758, 781, 806, 833, 881, 908, 966, 995, 1011, 1030, 1049, 1070, 1086, 1103, 1113, 1159, 1215, 1238, 1,2,5-5, 1273, 1315, 1367, 1377, 1387, 1410, 1460, 1481, 1549, 1585, 1605, 1689, 1730, 2617, 2872, 2926, 2939, 2974, 3088, 3144, 3437. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.74 (3H, s, Me-26), 0.79 (3H, s, Me-25), 0.80 (3H, s, Me-24), 0.83 (3H, d, $J_{29\text{-}19}$ = 6.2 Hz, Me-29), 0.91 (3H, s, Me-23), 0.93 (3H, s, Me-30), 1.05 (3H, s, Me-27), 2.16 (1H, d $J_{18\text{-}19}$ = 11.1 Hz, H-18), 2.56-2.66 (4H, m, 2H-2', 2H-3'), 4.42-4.49 (1H, m, H-3), 5.19-5.22 (1H, m, H-12), 5.22-5.26 (2H, m, 2H-5'), 5.85 (2H, c, 2H-8') 7.63 (2H, d, J = 8.6 Hz, H-13', H-15'), 7.83 (1H, s, H-7'), 7.87 (2H, d, J = 8.4 Hz, H-12', H-16'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.5 (C-25, q), 16.7 (C-24, q), 17.0 (C-26, q), 17.0 (C-29, q), 18.1 (C-6, t), 21.2 (C-30, q), 23.2 (C-11, t), 23.5 (C-2, t), 23.6 (C-27, q), 24.0 (C-16, t), 27.9 (C-15, t), 28.0 (C-23, q), 29.1 (C-2', t), 29.4 (C-3', t), 30.6 (C-21, t), 32.7 (C-7, t), 36.7 (C-22, t), 36.8 (C-10, s), 37.7 (C-1, t), 38.1 (C-4, s), 38.8 (C-20, d), 39.0 (C-19, d), 39.4 (C-8, s), 41.8 (C-14, s), 44.1 (C-8', t), 47.4 (C-9, d), 47.9 (C-17, s), 52.5 (C-18, d), 55.2 (C-5, d), 57.7 (C-5', t), 81.4 (C-3, d), 121.8 (C-11', s), 124.3 (C-7', d), 125.6 (C-12, d), 127.3 (C-14', s), 128.5 (C-12', d), 128.5 (C-16', d), 132.6 (C-13', d), 132.6 (C-15', d), 137.9 (C-13, s), 143.9 (C-6', s), 160.0 (C-9', s), 165.5 (C-10', s), 171.8 (C-4', s), 172.2 (C-1', s), 183.5 (C-28, s). Elemental analysis: found C 63.16%, H 6.91%, N 8.04%; calculated C 63.15%, H 6.91%, Br 8.13%, N 8.00%.

3β-(4-Oxo-4-((1-((5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-butanoyloxy)-urs-12-en-28-oic acid ***(11d)***

The title compound was prepared in the reaction of propargyl ester **10** with azide **3'd** as white powder in 38% yield. IR (KBr, cm^{-1}): 440, 501, 555, 569, 661, 677, 729, 758, 771, 810, 829, 889, 908, 924, 966, 995, 1034, 1049, 1093, 1159, 1240, 1,2,5-5, 1271, 1315, 1350, 1377, 1389, 1410, 1458, 1543, 1566, 1581, 1606, 1689, 1732, 2619, 2872, 2928, 2974, 3090, 3144, 3435. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.74 (3H, s, Me-26), 0.79 (3H, s, Me-25), 0.80 (3H, s, Me-24), 0.83 (3H, d, J_{29-19} = 6.4 Hz, Me-29), 0.91 (3H, s, Me-23), 0.93 (3H, s, Me-30), 1.05 (3H, s, Me-27), 2.16 (1H, d J_{18-19} = 11.1 Hz, H-18), 2.55-2.66 (4H, m, 2H-2', 2H-3'), 4.42-4.49 (1H, m, H-3), 5.19-5.23 (1H, m, H-12), 5.23-5.25 (2H, d, J = 4.2 Hz, 2H-5'), 5.86 (2H, c, 2H-8'), 7.58 (1H, dd, J_1 = 8.3, J_2 = 1.3 Hz, H-16'), 7.83 (1H, s, H-7'), 7.86 (1H, ddd, $J_1 = J_2$ = 2.0 Hz, H-12'), 8.02 (1H, ddd, $J_1 = J_2$ = 1.8 Hz, H-15'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 15.4 (C-25, q), 16.7 (C-24, q), 17.0 (C-26, q), 17.0 (C-29, q), 18.0 (C-6, t), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-2, t), 23.5 (C-27, q), 24.0 (C-16, t), 27.9 (C-15, t), 28.0 (C-23, q), 29.0 (C-2', t), 29.3 (C-3', t), 30.5 (C-21, t), 32.7 (C-7, t), 36.6 (C-22, t), 36.8 (C-10, s), 37.6 (C-1, t), 38.1 (C-4, s), 38.7 (C-20, d), 38.9 (C-19, d), 39.4 (C-8, s), 41.8 (C-14, s), 44.1 (C-8', t), 47.3 (C-9, d), 47.8 (C-17, s), 52.4 (C-18, d), 55.1 (C-5, d), 57.6 (C-5', t), 81.4 (C-3, d), 122.6 (C-11', s), 124.3 (C-7', d), 125.5 (C-12, d), 126.1 (C-16', d), 128.8 (C-12', d), 131.4 (C-15', d), 133.8 (C-14', s), 137.0 (C-13', s), 137.9 (C-13, s), 143.9 (C-6', s), 160.3 (C-9', s), 164.4 (C-10', s), 171.8 (C-4', s), 172.1 (C-1', s), 183.4 (C-28, s). Found, m/z: 863.3800 [M]+ $C_{46}H_{59}O_7N_5{}^{35}Cl_2$ Calculated, m/z: 863.3786

3β-(4-Oxo-4-((1-((4-methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-butanoyloxy)-urs-12-en-28-oic acid ***(12)***

The title compound was prepared in the reaction of propargyl ester **10** with azide **4'c** as off-white powder in 76% yield. IR (KBr, cm^{-1}): 571, 600, 660, 675, 771, 781, 793, 808, 829, 849, 881, 910, 924, 968, 991, 1020, 1045, 1103, 1159, 1188, 1203, 1,2,3-6, 1,2,5-5, 1271, 1315, 1369, 1389, 1423, 1458, 1520, 1610, 1691, 1732, 2621, 2874, 2928, 2943, 2974, 3144, 3435. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.75 (3H, s, Me-26), 0.81 (3H, s, Me-25), 0.82 (3H, s, Me-24), 0.83 (3H, d, J_{29-19} = 6.4 Hz, Me-29), 0.92 (6H, s, Me-23б Me-30), 1.05 (3H, s, Me-27), 2.16 (1H, d J_{18-19} = 11.4 Hz, H-18), 2.41 (3H, s, Me-10'), 2.58-2.66 (4H, m, 2H-2', 2H-3'), 4.45-4.51 (1H, m, H-3), 5.18-5.24 (3H, m, H-12, 2H-5'), 5.43 (2H, c, 2H-8'), 7.78 (1H, s, H-7'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 10.9 (C-11', q), 15.4 (C-25, q), 16.7 (C-24, q), 17.0 (C-26, q), 17.0 (C-29, q), 18.0 (C-6, q), 21.1 (C-30, q), 23.2 (C-11, t), 23.4 (C-2, t), 23.5 (C-27, q), 23.9 (C-16, t), 27.9 (C-15, t), 29.0 (C-2', t), 29.3 (C-3', t), 30.5 (C-23, q), 32.7 (C-21, t), 36.6 (C-7, t), 36.8 (C-22, t), 37.6 (C-10, s), 38.1 (C-1, t), 38.7 (C-4, s), 38.8 (C-20, d), 38.9 (C-19, d), 39.4 (C-8, s), 41.3 (C-8', t), 41.8 (C-14, t), 47.3 (C-9, d), 47.8 (C-17, s), 52.4 (C-18, d), 55.1 (C-5, d), 57.5 (C-5', d), 81.3 (C-3, d), 111.9 (C-10', s), 124.3 (C-7', s), 125.6 (C-12, d), 137.9 (C-13, s), 143.9 (C-6', s), 154.0 (C-9', s), 171.8 (C-4', s), 172.1 (C-1', s), 183.9 (C-28, s). Elemental analysis: found C 65.71%, H 7.87%, N 9.29%; calculated C 65.66%, H 7.93%,, N 9.34%.

(1-((4-Methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl 3-oxo-lup-20(29)-en-28-oate ***(14a)***

The title compound was prepared in the reaction of propargyl ester **13a** with azide **4'** as white powder in 75% yield. IR (cm^{-1}): 444, 500, 544, 582, 600, 652, 669, 702, 789, 849, 883, 916, 939, 966, 989, 1009, 1024, 1045, 1084, 1126, 1140, 1173, 1228, 1281, 1294, 1317, 1352, 1379, 1458, 1520, 1552, 1610, 1641, 1703, 1724, 2870, 2949, 3072, 3145, 3435. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, Me-) 0.88 (3H, s), 0.92 (3H, s), 0.99 (3H, s), 1.03 (3H, s), 1.65 (3H, s, Me-30), 2.43 (3H, s, Me-7'), 2.91-3.01 (1H, m, H-19),), 4.58 (1H, br.s, H-30), 4.71 (1H, br.s, H-30), 5.18 (2H, AB, J_{AB} = 12.4 Hz, Δη = 18.9, 2H-1'), 5.42 (2H, AB, J_{AB} = 12.1 Hz, Δη = 8.7, 2H-4'), 7.79 (1H, C, H-3'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 11.0 (C-7', q), 14.5 (C-27, q), 15.2 (C-25, q), 15.9 (C-26, q), 19.3 (C-30, q), 19.5 (C-6, t), 20.9 (C-24, q), 21.3 (C-11, t), 25.4 (C-12, t), 26.5 (C-23, q), 29.5 (C-15, t), 30.4 (C-21, t), 31.8 (C-16, t), 33.4 (C-7, t), 34.1 (C-2, t), 36.8 (C-10, s), 36.8 (C-22, t), 38.2 (C-13, d), 39.5 (C-1, t), 40.5 (C-8, s), 41.2 (C-4', t), 42.3 (C-14, c), 46.8 (C-19, d), 47.2 (C-4, c), 49.2 (C-9, d), 49.8 (C-18, d), 54.8 (C-5, d), 56.4 (C-17, c), 56.8 (C-1', t), 109.7 (C-29, t), 111.8 (C-6', s), 124.5 (C-3', d), 144.2 (C-2', s), 150.2 (C-20, s), 153.9 (C-5', s), 175.9 (C-28, s), 218.1 (C-3, c). Found, m/z: 647.4035 $[M]+$ $C_{37}H_{53}O_5N_5$ Calculated, m/z: 647.4041.

(1-((4-Methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl-3β-hydroxy-lup-20(29)-en-28-oate ***(14b)***

The title compound was prepared in the reaction of propargyl ester **13b** with azide **4'** as off-white powder in 87% yield. IR (cm^{-1}): 544, 600, 652, 669, 789, 849, 885, 914, 945, 984, 1009, 1043, 1074, 1107, 1134, 1151, 1174, 1230, 1271, 1294, 1319, 1360, 1377, 1389, 1454, 1520, 1608, 1641, 1726, 2870, 2943, 3072, 3145, 3439. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.67 (3H, s, Me-24), 0.70 (3H, s, Me-26), 0.73 (3H, s, Me-25), 0.91 (3H, s, Me-27), 0.93 (3H, s, Me-23), 1.64 (3H, C, Me-29), 2.43 (3H, C, Me-7'), 2.90-2.99 (1H, m, H-19),),3.11-3.18 (1H, m, H-3), 4.57 (1H, br.s, H-29), 4.70 (1H, br.s, H-29), 5.18 (2H, AB, J_{AB} = 12.7 Hz, Δη = 20.3, 2H-1'), 5.41 (2H, AB, J_{AB} = 11.5 Hz, Δη = 10.0, 2H-4'), 7.79 (1H, s, H-3'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 11.0 (C-7', q), 14.6 (C-27, q), 15.3 (C-24, q), 15.4 (C-25, q), 16.1 (C-26, q), 18.1 (C-6,), 19.3 (C-30, q), 20.7 (C-11, t), 25.4 (C-12, t), 27.3 (C-2, t), 27.9 (C-23, q), 29.5 (C-21, t), 30.4 (C-15, t), 31.9 (C-16, t), 34.1 (C-22, t), 36.8 (C-7, t), 37.1 (C-10, s), 38.2 (C-13, d), 38.6 (C-1, t), 38.8 (C-4, s), 40.6 (C-8, s), 41.2 (C-4', t), 42.2 (C-14, s), 46.8 (C-19, d), 49.3 (C-18, d), 50.4 (C-9, d), 55.2 (C-5, d), 56.4 (C-17, s), 56.7 (C-1', t), 78.8 (C-3, d), 109.6 (C-29, d), 111.8 (C-6', s), 124.6 (C-3', d), 144.2 (C-2', s), 150.2 (C-20, s), 153.9 (C-5', s), 175.9 (C-28, s). Found, m/z: 649.4193 $[M]^+$ $C_{37}H_{55}O_5N_5$ Calculated, m/z: 649.4198.

(1-((5-Phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-3-oxo-lup-20(29)-en-28-oate ***(15a)***

The title compound was prepared in the reaction of propargyl ester **13b** with azide **4'** as off-white powder in 66% yield. IR (KBr, cm^{-1}): 434, 442, 498, 519, 544, 582, 665, 690, 710, 756, 802, 885, 918, 935, 964, 985, 1016, 1047, 1070, 1086, 1126, 1140, 1153, 1173, 1215, 1227, 1292, 1317, 1350, 1379, 1452, 1483, 1552, 1578, 1591, 1610, 1641, 1703, 1724, 1778, 2870, 2949, 3070, 3145, 3435. NMR ^{1}H (400.13 MHz , $CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, Me-25) 0.83 (3H, s, Me-26), 0.90 (3H, s, Me-27), 0.97 (3H, s, Me-24), 1.02 (3H, s, Me-23), 1.60 (1H,t, $J_{18\text{-}19}$ = 11.3 Hz, H-18), 1.63 (3H, s, Me-30), 2.89-2.98 (1H, m, H-19), 4.56 (1H, br.s, H-29), 4.69 (1H, br.s, H-29), 5.23 (2H, AB-sys, J_{AB} = 12.0 Hz, Δη = 14.2, 2H-4'), 5.89 (2H, AB-sys, J_{AB} = 13.8 Hz, Δη = 14.8, 2H-1'), 7.46-7.58 (3H, m, H-9', H-10', H-11'), 7.93 (1H, s, H-3'), 7.97-8.03 (2H, m, H-8', H-12'). NMR ^{13}C (125.76 MHz , $CDCl_3$, δ, ppm): 14.45 (C-27, q), 15.15 (C-25, q), 15.80 (C-26, q), 19.2 (C-30, q), 19.4 (C-6, t), 20.9 (C-24, q), 21.2 (C-11, t), 25.3 (C-12, t), 26.4 (C-23, q), 29.4 (C-15, t), 30.3 (C-21, t), 31.7 (C-16, t), 33.4 (C-7, t), 34.0 (C-2, t), 36.7 (C-10, s), 36.7 (C-22, t), 38.2 (C-13, d), 39.4 (C-1, t), 40.4 (C-8, s), 42.2 (C-14, s), 44.1 (C-4', t), 46.7 (C-19, d), 47.2 (C-4, s), 49.1 (C-9, d), 49.7 (C-18, d), 54.8 (C-5, d), 56.3 (C-17, s), 56.8 (C-1', t), 109.7 (C-29, t), 122.8 (C-7', s), 124.4 (C-3', d), 127.0 (C-8', d), 127.0 (C-12', d), 129.1 (C-9', d), 129.1 (C-11', d), 132.3 (C-10', d), 144.2 (C-2', s), 150.1 (C-20, s), 159.8 (C-5', s), 166.0 (C-6', s), 175.8 (C-28, s), 217.9 (C-3, s). Calculated m\z: 693.4259 Found m\z: 693.4252.

Synthesis of acetates 8b, 9b, 14c

To a stirred mixture of **8a** or **9a or 14b** (1 mmol), Et_3N (2.0 g, 20 mmol) in CH_2Cl_2 (20 mL) acetic anhydride (1.0 g, 10 mmol) was added at room temperature. The mixture was stirred overnight (TLC control) after which MeOH (1.0 g, 30 mmol) was added. The resulting mixture was washed with HCl_{aq} (3%, 2×20 mL), $NaHCO_{3\ aq}$ (5%, 20 mL), dried (Na_2SO_4) and concentrated. The residue was chromatographed (SiO_2, CH_2Cl_2, MTBE) to afford acetates **9a** or **9b** or **17.**

(1-((4-Methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl-3β-acetoxy-urs-12-en-28-oate ***(8b)***

The title compound was prepared in the acylation reaction of compound **9a** as white powder in 90% yield. IR (KBr, cm^{-1}): 602, 652, 660, 700, 733, 756, 791, 829, 849, 906, 916, 953, 987, 1028, 1043, 1078, 1113, 1138, 1167, 1178, 1194, 1248, 1308, 1371, 1390, 1456, 1520, 1610, 1659, 1732, 1765, 2874, 2929, 2949, 2968, 3145, 3435. NMR ^{1}H (400.13 MHz , $CDCl_3$**,** δ, ppm, J/Hz**):** 0.69 (3H, s, Me-26), 0.82 (3H, s, Me-25), 0.84 (6H, s, Me-29, Me-24), 0.91 (3H, s, Me-30), 0.93 (3H, s, Me-23), 1.05 (3H, s, Me-27), 2.01 (4H, s, Me-2"), 2.20 (1H, d, J=11.2 Hz, H-18), 2.42 (3H, s, Me-9'), 4.43-4.57 (3H, m, H-3 + 2H-1'), 5.19-5.22 (1H, ddd, J_1=J_2 = 3.4 Hz, H-12), 5.24 (2H, s), 5.37 (2H, s, 2H-4'), 7.77 (1H, s, H-6'). NMR ^{13}C (125.76 MHz , $CDCl_3$**,** δ, ppm, J/Hz**)**: 10.9 (C-7', q), 15.4 (C-25, q), 16.7 (C-24, q), 16.7 (C-26, q), 16.9 (C-29, q), 18.1 (C-6, t), 21.1 (C-30, q), 21.2 (C-2", q), 23.2 (C-11, t),

23.4 (C-27, q), 23.5 (C-16, t), 24.1 (C-2, t), 27.9 (C-15, t), 28 (C-23, q), 30.5 (C-21, t), 32.8 (C-7, t), 36.5 (C-22, t), 36.8 (C-10, s), 37.6 (C-1, t), 38.2 (C-4, s), 38.7 (C-20, d), 39 (C-19, d), 39.4 (C-8, s), 41.2 (C-4', t), 42 (C-14, s), 47.3 (C-9, d), 48.1 (C-17, s), 52.7 (C-18, d), 55.2 (C-5, d), 57.1 (C-1', t), 80.8 (C-3, d), 111.8 (C-6', s), 124.5 (C-3', d), 125.5 (C-12, d), 137.9 (C-13, s), 144.2 (C-2', s), 153.9 (C-5', s), 170.9 (C-1", s), 177.3 (C-28, s). Found, m/z: 691,4311 [M]+ $C_{39}H_{57}O_6N_5$ Calculated, m/z: 691,4302.

3-((4-((3β-Acetoxy-urs-12-en-28-oyl-oxyacetoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-methyl-1,2,5-oxadiazole 2-oxide ***(9b)***The title compound was prepared in the acylation reaction of compound **9a** as white powder in 86% yield. IR (KBr, cm^{-1}): 600, 661, 679, 700, 733, 795, 806, 829, 849, 903, 922, 953, 970, 987, 1007, 1028, 1078, 1109, 1140, 1167, 1180, 1198, 1248, 1308, 1371, 1389, 1456, 1520, 1610, 1659, 1730, 2874, 2928, 2949, 2966, 3147, 3435. NMR ^1H (400.13 MHz , $CDCl_3$**,** δ, ppm, J/Hz): 0.55 (3H, s, Me-26), 0.81-0.85 (9H, m, Me-25, Me-29, Me-24), 0.87-0.92 (6H, m, Me-30, Me-23), 1.03 (3H, s, Me-27), 2.02 (3H, s, Me-2"), 2.18 (1H, d, O = 11.6 Hz, H-18), 2.41 (3H, s, Me-9'), 4.43-4.50 (1H, m, H-3), 5.12 (2H, AB, J_{AB} = 9.5 Hz, Δη = 12.7, 2H-1'), 5.17-5.21 (1H, m, H-12), 5.40 (2H, s, 2H-4'), 7.73 (1H, s, H-3') NMR ^{13}C (125.76 MHz , $CDCl_3$**,** δ, ppm, J/Hz**)**: 11.0 (C-9', q), 15.5 (C-25, q), 16.7 (C-24, q), 16.9 (C-26, q), 17.0 (C-29, q), 18.1 (C-6, t), 21.1 (C-30, q), 21.3 (C-2", q), 23.2 (C-11, t), 23.4 (C-27, q), 23.5 (C-16, t), 24.1 (C-2, t), 27.9 (C-15, t), 28.0 (C-23, q), 30.6 (C-21, t), 32.9 (C-7, t), 36.4 (C-22, t), 36.8 (C-10, s), 37.6 (C-1, t), 38.2 (C-4, s), 38.7 (C-20, d), 39 (C-19, d), 39.5 (C-8, s), 41.3 (C-6', t), 42 (C-14, s), 47.4 (C-9, d), 48.1 (C-17, s), 52.6 (C-18, d), 55.2 (C-5, d), 57.8 (C-3', t), 60.2 (C-1', t), 80.9 (C-3, d), 111.9 (C-7', s), 124.6 (C-5', d), 125.6 (C-12, d), 137.8 (C-13, s), 143.3 (C-4', s), 154.0 (C-8', s), 167.9 (C-2', s), 171.0 (C-1", s), 176.9 (C-28, s). Found, m/z: 749.4367 [M]+ $C_{41}H_{59}O_8N_5$ Calculated, m/z: 749.4358.

(1-((4-Methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl 3-acetoxy-lup-20(29)-en-28-oate ***(14c)***

The title compound was prepared in the acylation reaction of compound **9a** as white powder in 92% yield. IR (KBr, cm^{-1}): 490, 544, 600, 652, 667, 700, 756, 789, 849, 885, 916, 980, 1028, 1041, 1107, 1130, 1149, 1196, 1250, 1317, 1375, 1390, 1454, 1479, 1520, 1556, 1610, 1641, 1726, 2727, 2872, 2947, 3072, 3145. NMR ^1H (400.13 MHz , $CDCl_3$**,** δ, ppm, J/Hz): 0.67 (3H, s, Me-25), 0.81 (9H, m, Me-24, Me-26, Me-27), 0.90 (3H, s, Me-23), 1.60 (1H, m, H-18), 1.65 (3H, s, Me-30), 2.01 (3H, s, Me-2"), 2.03-2.13 (1H, m, H-15), 2.43 (3H, s, Me-7'), 2.90-3.00 (1H, td, J_1 = 10.8 Hz, J_2 = 4.6 Hz, H-19), 4.39-4.47 (1H, m, H-3), 4.58 (1H, br.s., H-29), 4.71 (1H, br,s., H-29), 5.18 (2H, AB-sys, J_{AB} = 12.9 Hz, Δη = 19.66, 2H-1'), 5.41 (2H, AB-sys, J_{AB} = 10.6 Hz, Δη = 9.76, 2H-4'), 7.79 (1H, s, H-3'). NMR ^{13}C (125.76 MHz , $CDCl_3$**,** δ, ppm, J/Hz**)**: 11.0 (C-7', q), 14.6 (C-27, q), 15.4 (C-24, q), 16.1 (C-25, q), 16.4 (C-26, q), 18.1 (C-6, t), 19.3 (C-30, q), 20.8 (C-11, t), 21.3 (C-2", q), 23.6 (C-12, t), 25.4 (C-2, t), 27.9 (C-23, q), 29.5 (C-21, t), 30.4 (C-15, t), 31.9 (C-16, t), 34.1 (C-22, t), 36.8 (C-7, t), 37.0

(C-10, s), 37.7 (C-4, s), 38.2 (C-13, d), 38.3 (C-1, t), 40.6 (C-8, s), 41.2 (C-4', t), 42.3 (C-14, s), 46.8 (C-19, d), 49.3 (C-18, d), 50.3 (C-9, d), 55.3 (C-5, d), 56.5 (C-17, s), 56.8 (C-1', t), 80.9 (C-3, d), 109.7 (C-29, d), 111.8 (C-6', s), 124.5 (C-3', d), 144.3 (C-2', s), 150.3 (C-20, s), 153.9 (C-5', s), 171.0 (C-1", s), 175.9 (C-28, s). Found, m/z: 691.4303 [M]+ $C_{39}H_{57}O_6N_5$ Calculated, m/z: 691.4304.

2.1.3. Cytotoxicity of triterpenoid-azole hybrids

DMEM with 10% fetal bovine serum (Gibco, USA) was used for culturing. Optical density was measured on a Multiskan RC spectrophotometer (ThermoFischer Scientific, USA). Cultures of breast cancer MCF7 (ATCC number HTB-22), U-87 MG glioblastoma multiform cells (ATCC number HTB-14), A549 lung carcinoma (ATCC number CRM-CCL-18), HepG2 hepatocarcinoma, (ATCC number HB-8065) were obtained commercially (ATCC, USA). The noncancer control was immortalized human fibroblasts (ATCC number CRL-4058). Cytotoxicity of the tested compounds was assessed using the MTT assay and the standard procedure [40]. Cells were inoculated into 96-well plates (3,000 cells per well) and incubated at 37°C in 5% CO_2 for attachment. Medium in wells was replaced after 24 h with fresh medium containing the tested compounds in DMSO (1 % v/v) and incubated for 72 h. Optical density was measured in a plate spectrophotometer as usual. All compounds were tested at concentrations of 10, 25, 50, and 100 μM using the required controls, i.e., negative, DMSO (solvent), and positive, doxorubicin (standard cytostatic). Each experiment was performed independently in triplicate with three tests in each. Statistical processing of the results was performed using the Microsoft Excel-2007, STATISTICA 6.0 and GraphPad Prism 5.0 programs. Results were reported as mean inhibitory concentration IC_{50} ± SEM. Reliability of differences (p) was estimated using the Student t test. The differences with $p < 0.05$ were considered as reliable.

2.1.4. Flow cytometry analysis

For apoptosis and cell cycle analysis two different types of MCF7 cell line was used: wild type (p53 +/+) and p53-deficient (p53 -/-), kindly provided by P.N. Chumakov (Engelhardt Institute of Molecular Biology RAS, Russia). Cells were cultivated on DMEM with 10% fetal bovine serum (Gibco, USA). 48 h before experiment cells were seeded into 6-well plates (30000 cells per plate) and incubated 24 h at 37°C in 5% CO2 for attachment. Then medium in wells was replaced with fresh medium containing the tested compounds in GI50 concentration and incubated for 24 h followed by flow cytometry analysis. Also induction for overexpression of p53 protein was performed using an 10 nM estrogen (Sigma) treatment.

For apoptosis detection FITC Annexin V Apoptosis Detection Kit I was used (BD Pharmingen). For investigation of cell cycle Hoechst 33342 Ready Flow Reagent was used (Invitrogen, ThermoFisher Scientific). Sample preparation was performed according to the protocol, doxorubicin as cytostatic and

nutlin-3 (Calbiochem) as MDM2 inhibitor were used as positive controls. Analysis performed on at least 30000 cells. CytoFLEX flow cytometer (Beckman Coulter) was used for analysis. The results are presented as a percentage of cell population standing in different stages of the cell cycle.

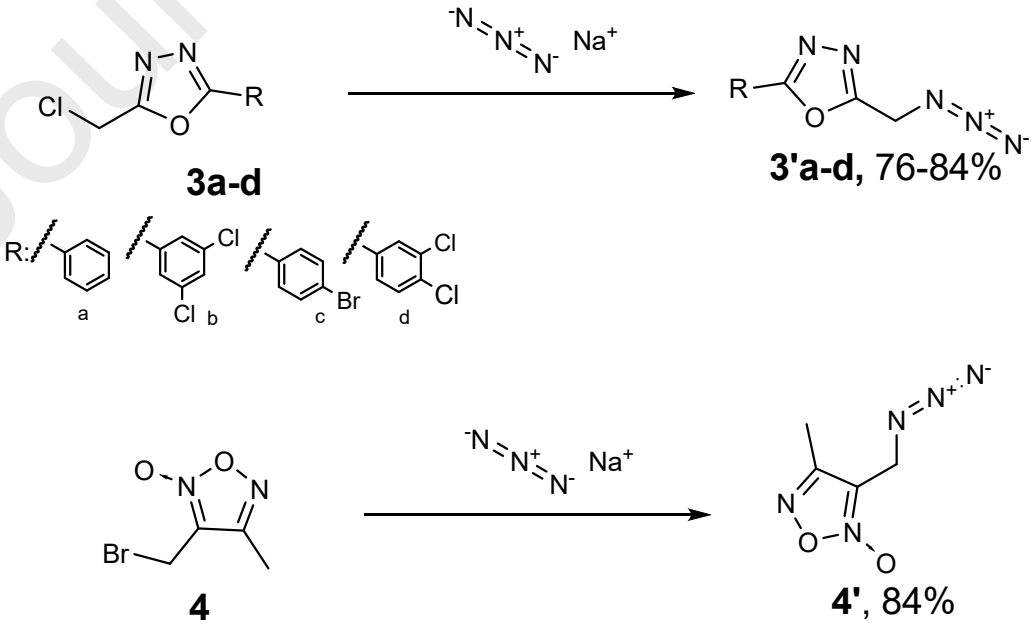
2.1.5. Molecular docking methods

Molecular modeling was carried out in the *Schrodinger Maestro* visualization environment using applications from the *Schrodinger Small Molecule Drug Discovery Suite 2016-1* package [41]. Three-dimensional structures of the derivatives were obtained empirically in the *LigPrep* application using the OPLS3 force field [42]. For the calculations, the XRD model of Mdm2 protein with PDB ID 5OAI [43] (resolution 2 Å) was chosen. To model a possible mechanism of inhibition of selected target, molecular docking of new compounds was performed at the binding site of 3-[(1R)-2-(Tert-butylamino)-1-[formyl-[(3,4,5-trifluorophenyl)methyl]amino]-2-oxoethyl]-6-chloro-1H-indole-2-carboxylic acid (B5K) using *Glide* [44]. The search area for docking was selected according to the size of known inhibitor. Docking was performed in comparison with the known inhibitors B5K and nutlin-3A. The three-dimensional structures of inhibitors were obtained in the PubChem database and prepared in the *LigPrep* application. Non-covalent interactions of compounds in the binding site were visualized using *Schrodinger Maestro*.

3. Results and discussion

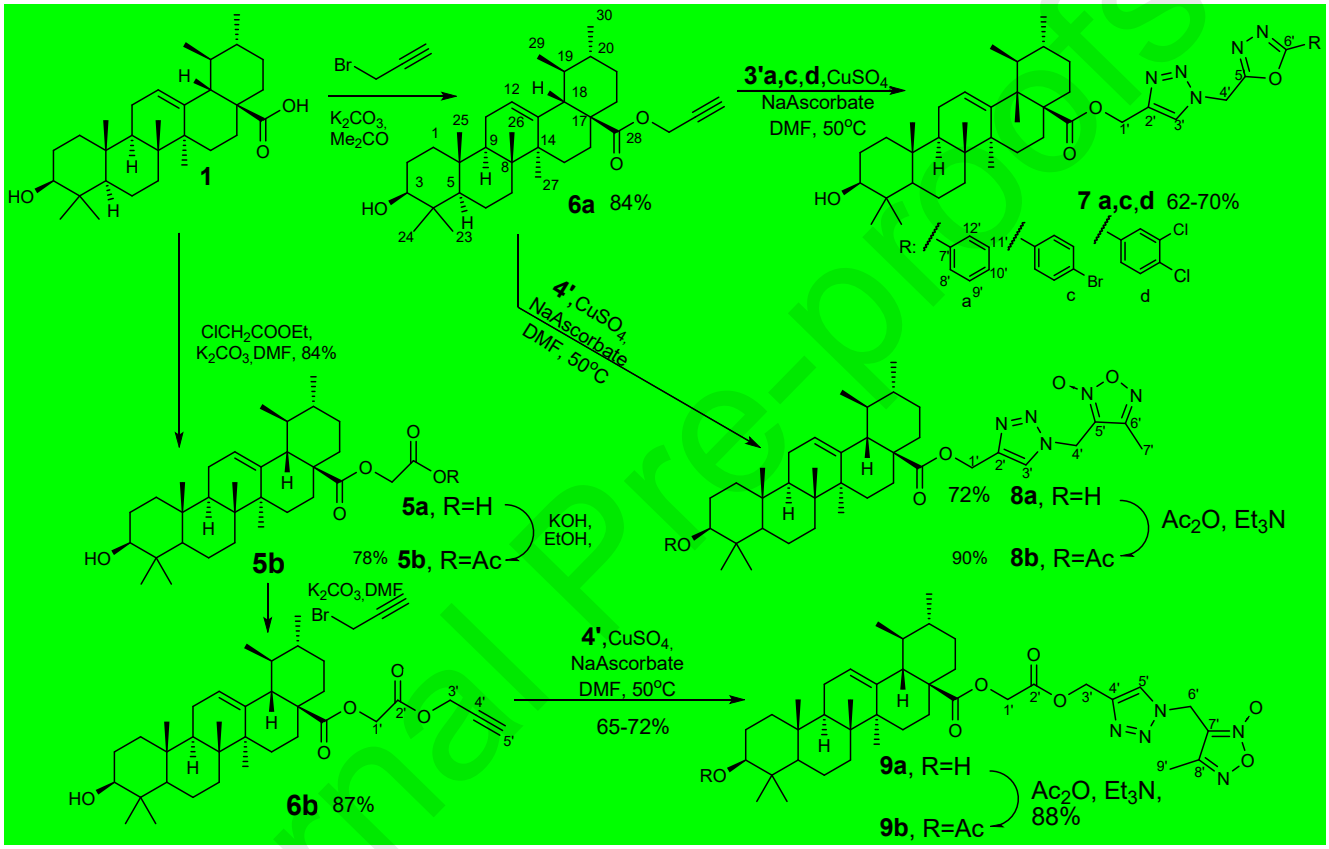
3.1. Chemistry

The synthetic pathways to the intermediate compounds and new hybrid derivatives are depicted on the schemes 1-4.



Scheme 1. Synthesis of azide derivatives from 2-(chloromethyl)-5-aryl-1,3,4-oxadiazoles and 3-(bromomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide.

2-(azidomethyl)-5-aryl-1,3,4-oxadiazoles **3'a-d** were prepared by the reaction of corresponding chloromethyl derivatives **3a-d** with the excess of NaN_3 in dimethylformamide (76-84% yield, **Scheme 1**). Similarly to it, 3-(azidomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide **4'** was obtained from bromomethyl derivative **4** in 84% yield.



Scheme 2. Synthesis of ursolic acid C-28 ester conjugates with 5-aryl-1,3,4-oxadiazoles and 4-methyl-1,2,5-oxadiazole 2-oxide tethered with 1H-1,2,3-triazol-4-yl)methyl- linkers.

Synthesis of propargyl esters of triterpenoids

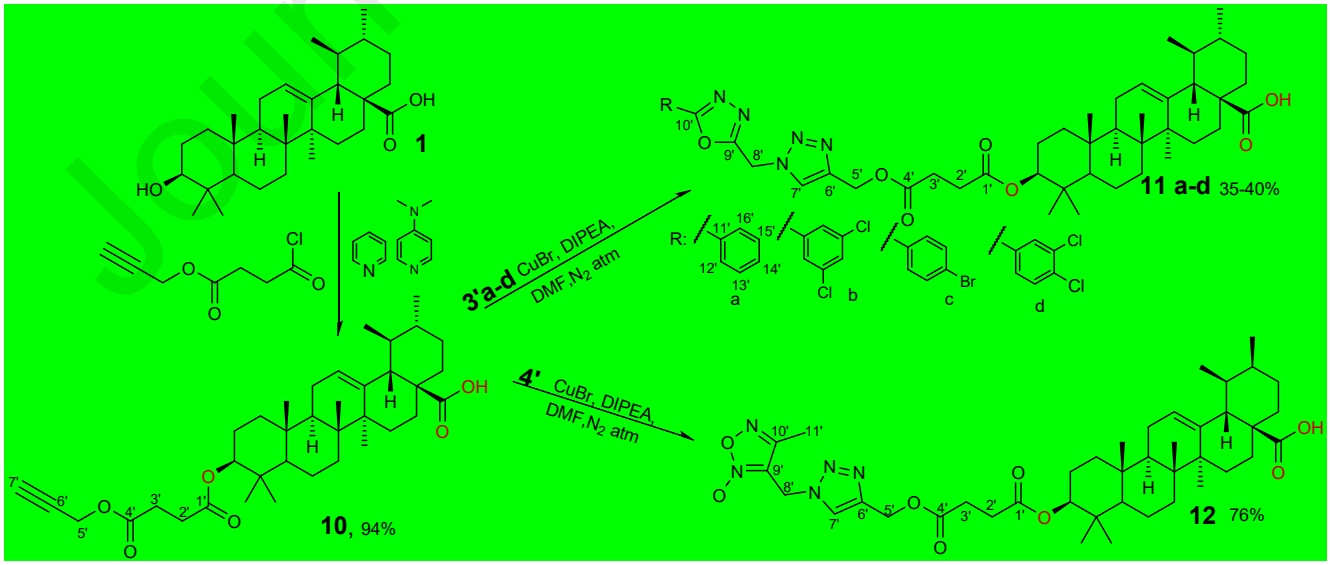
Alkylation of ursolic acid with propargyl bromide in acetone in the presence of K_2CO_3 afforded propargyl ursolate **6a** in 84% yield (**Scheme 2**). Similarly, propargyl betulonate **2a** was prepared by alkylation of betulonic acid **2** in 82% yield (**Scheme 4)**.

Compound **6b** was synthesized in 3 steps (alkylation-hydrolysis-propargylation) in general 57% yield starting from ursolic acid **1**. (**Scheme 2**). Alkylation of ursolic acid **1** with ethyl chloroacetate resulted in ursolic acid carboxymethyl ester. The terminal ester group of **5a** was hydrolyzed selectively

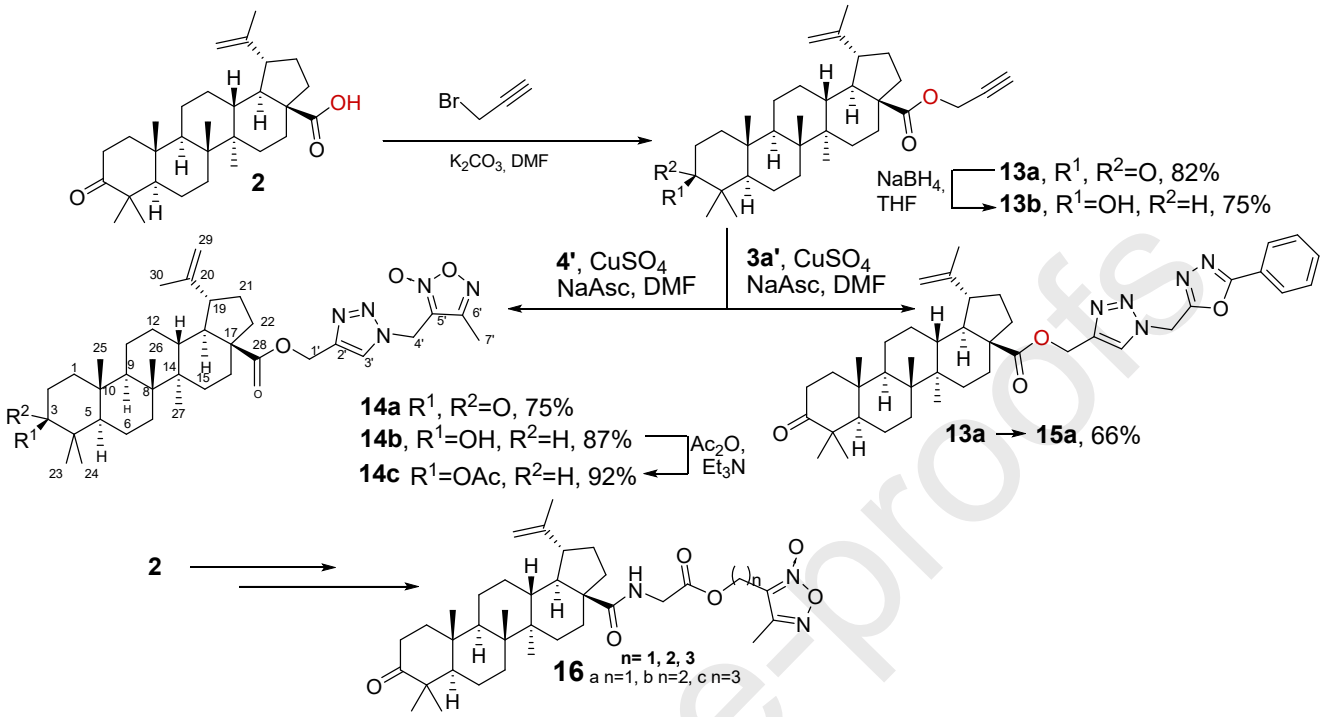
following with the known method [45]. The alkylation of acid **5b** was carried out in DMF to give compound **6b** with propargyl ester group tethered by the additional ester-type linker from ursane core (87%). Certain ursolic acid hybrids including 3-O-succinyl linker and terminal 1,2,5- oxadiazole group displayed notable cytotoxic activity [46]. Therefore, we were interested in the preparation of ursane derivatives with a free C-28 carboxyl group and heterocyclic combinations attached at the 3-O-position on the triterpene core. Ursolic acid (4-propargyl)-succinate **10** was prepared by acylation of **1** with prop-2-ynyl 4-chloro-4-oxobutanoate in the presence of pyridine and 4-DMAP in 78% yield (**Scheme 3**). Propargyl betulonate **13a** was prepared from betulonic acid **2a** in 88% yield (**Scheme 4**). Reduction of **13a** with $NaBH_4$ following the previously published method [35] resulted in propargyl betulinate **13b** in 75% yield.

Synthesis of triterpenoid-triazole-oxadiazole hybrids.

Ursane and lupane type (1-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl esters and (1-((4-Methyl-2-oxido-1,2,5-oxadiazol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl esters were prepared by 1,3-cycloaddition reactions of corresponding azole-derived azides with propargyl esters tailored to C-3 or C-28 position of triterpenoids. Propargyl esters **6a**, **6b** reacted with azides **3'a-d**, **4'** in DMF at 50ºC in the presence of $CuSO_4$ and sodium ascorbate (**Scheme 2**) to afford triple hybrids **7a-d** (62-70%), **8a**, **9a** (65-72%). Lupane-type hybrids **14 a,b**, **15a** were prepared similarly to above mentioned from the esters **13 a,b** in 82-84% yield (**Scheme 4**). In the specified conditions, the reactions of propargyl succinate **10** with azides were sluggish and the yields of hybrids **11**, **12** were low. Therefore, the interaction of **10** with azides **3'a-d**, **4'** was carried out in DMF under N_2 atmosphere at ambient temperature using a combination of CuBr-DIPEA as a catalyst. In this way, compounds **11a-d** and **12** were prepared in 35-40% and 75% yield (**Scheme 3**).



Scheme 3. Synthesis of ursolic acid C-3 ester conjugates with 5-aryl-1,3,4-oxadiazoles and 4-methyl-1,2,5-oxadiazole 2-oxide tethered with 4-(1H-1,2,3-triazol-4-yl)methoxy)-4-oxobutanoate- linkers.



Scheme 4. Synthesis of lupane-type conjugates with 5-aryl-1,3,4-oxadiazoles and 4-methyl-1,2,5-oxadiazole 2-oxide.

The structures of the new compounds were elucidated by NMR, IR, MS, and elemental analysis (**11b**, **11c**, **12**) data. In the ^{1}H NMR spectra of the triazolo-oxadiazole derivatives, a triazole proton as a singlet was observed in the region of 7.8 ppm. For hybrid triazole-1,3,4-oxadiazoles **7a**,**c**,**d**, **11 a-d** and triazole-furoxan hybrids **8a**,**b**, **9a**,**b**, **12**, **14a**,**b**,**c** the signals of O-C$\underline{H}_2$-triazole were registered at 5.08-5.25 ppm (^{1}H spectra) and 57.1-57.8 (^{13}C spectra). The corresponding signals of triazole-C$\underline{H}_2$-oxadiazole were found for 1,3,4-oxadiazole compounds at 5.30-5.86 ppm (^{1}H) and 44.0-44.1 (^{13}C) and for furoxan derivatives 5.40 (^{1}H) and 41.3 (^{13}C). The methylene protons of the linker group O-C$\underline{H}_2$-COOCH_2- for compounds **9a**,**b** were observed at 4.50 (^{1}H) and 60.2 ppm (^{13}C). In the ^{13}C spectra of hybrids of triazoles with 1,3,4 oxadiazoles **7a**,**c**,**d**, **11 a-d**, and triazoles with furoxan **8a**,**b**, **12**, **14a**,**b**,**c** signals of carbon atoms pair of the 1,2,3 triazoles were observed at 124.2-124.5; 143.8-144.3 ppm, while for furoxan derivatives **9a**,**b** with additional ester linkage signals of carbons of triazole moiety were 124.5; 143.3 ppm. The derivatives of **7a**,**c**,**d**, **11 a-d** showed characteristic signals of 1,3,4-oxadiazole carbon atoms at 159.8-160.5 and 164.4-166.1 ppm. The corresponding pair of carbon signals of furoxan moiety in compounds **8a**,**b**, **9a**,**b**, **12**, **14a**,**b** was registered at 111.8-111.9 and 153.9-154.0 ppm.

3.2. Cytotoxicity of hybrid compounds

Cytotoxicity of the novel compounds was assessed using the MTT assay towards breast cancer MCF7, glioblastoma multiform cells U-87 MG, lung carcinoma A549, hepatocarcinoma U-87 MG, using immortalized human fibroblasts as non-cancer control (Table 1).

Table 1. Concentrations of half-maximal inhibition (IC_{50} ± SEM, μM) of test compounds on immortalized human fibroblasts, MCF-7, U-87 MG, A549, HepG2 cells.

Compound*	immortalized human fibroblasts	MCF7	U-87 MG	A549	HepG2
Doxorubicin	3.33±0.67	4.51±1.12	2.05±0.22	6.17±1.17	10.02±1.67
Ursolic acid **1**	90.89±5.5	25.05±3.17	43.82±3.88	41.02±3.77	37.28±5.02
Betulonic acid **2**	16.7±2.99	28.78±4.12	45.12±6.78	10.91±1.13	45.0±6.7
8a	25.85±3.04	22.9±10.02	29.71±6.52	>100	12.89±2.63
8b	10.41±1.25	1.55±0.08	>100	>100	>100
9a	82.59±16.87	26.23±8.76	>100	>100	35.58±8.83
11a	74.9±4.58	>100	>100	40.26±7.55	>100
12	48.35±11.47	>100	81.67±2.89	>100	>100
14a	17.48±2.11	>100	>100	>100	54.13±7.05
14b	22.46±2.50	13.38±5.69	>100	>100	>100
14c	33.16±2.77	45.21±6.18	36.33±5.11	41.08±7.06	88.27±9.71

* Compounds **7a**, **7c**, **7d**, **9b**, **11b**, **11c**, **11d**, **11e**, **11f**, **15a**, **16a**, **16b**, **16c** were found non-active (IC_{50} >100 μM for all cells tested).

For the starting compounds ursolic acid **1** and betulonic acid **2**, cytotoxicity values were comparable towards MCF-7, U-87-MG, and HepG2 cells, while betulonic acid **2** exhibited a significantly higher cytotoxicity with respect to non-cancer cells and A-549 cells. The cytotoxicity of doxorubicin significantly exceeded the activity of the starting acids **1**,**2** for all studied cells. All hybrid derivatives, including a combination of 1,3,4-oxadiazoles and 1,2,3-triazoles, attached to the 28- position of ursane (**7a**, **7c**, **7d**) and lupane backbone (**15a**) were found non-active towards all the tested cells. Also, no cytotoxicity was revealed for the majority of double heterocyclic hybrids of 1,3,4-oxadiazoles and triazole, connected to position 3- of the ursane backbone *via* succinate linker (**11b**, **11c**, **11d**). In this series, only compound **11a**, including a combination of 5-phenyl-1,3,4-oxadiazole and 1,2,3-triazole, was slightly toxic towards to lung cancer cells line A-549, which was close to the activity of the starting ursolic acid **1**. The introduction of a furoxan fragment, attached *via* 1,2,3-triazole linker to the

triterpene backbone, led in certain cases to compounds with marked cytotoxic activity. Compound **8a** that was a combination of triazole and furoxan groups tailored to ursane C-28 position, showed cytotoxicity comparable to the starting compound **1 (**MCF-7 cells), whereas the superior activity comparing **1** was obtained towards U-87-MG cells. Concerning HepG2, the activity value of hybrid **8a** (12.89±2.63) was comparable with the data obtained for doxorubicin (10.02±1.67). At the same time, compound **8a** significantly exceeded doxorubicin in terms of the selectivity index (2.0 *vs* 0.33), respectively. The introduction of the additional ester spacer between the triterpene backbone and triazole fragment (compound **9a**) resulted in activity decrease against all the tested cells. The cytotoxicity of **9a** against MCF-7, HepG2 was close to the data obtained for ursolic acid **1**. Hybrid compound **12**, including a combination of 1,2,3-triazole and 1,2,5-oxadiazole, connected *via* a succinate linker to position C-3 of the ursane backbone, was inactive to all the cancer cells studied, while being more toxic against non-cancerous cells than ursolic acid **1**. It is known that the introduction of a 3-O-acetyl group into a triterpenoid molecule may result in the enhancement of cytotoxicity properties[14]. However, 3-O-acetate **9b** turned out to be completely non-toxic for all studied cells, in contrast to the initial 3-hydroxy derivative **9a**. The acetate derivative **8b** obtained from **8a** containing 3-hydroxyl group, was significantly less active than the parent compound against U-87-MG, HepG2, and A-549 cells. Surprisingly, in relation to MCF7 breast cancer cells, acetate **8b** demonstrated an excellent activity (1.55±0.08) which was superior over that for doxorubicin (4.51±1.12). The selectivity index of **8b** for MCF7 (6.72) also significantly exceeded the values obtained for ursolic acid (3.68) and doxorubicin (0.74). Hybrid of betulonic acid with furoxan and 1,2,3-triazole **14a** was found non-efficient towards cancer cells MCF-7, U-87 MG, A549, while it demonstrated toxicity similar to those of betulonic acid **2a** towards HepG2 and non-cancer cells. Therefore, ketoester **13a** was reduced with $NaBH_4$ to prepare the C-3-OH propargyl ester **13b** which was converted to lupane-type furoxan hybrid **14b**. Similarly to ursane analog **8a**, hydroxyl-bearing **14b** comprising a succession of 1,2,3-triazole and 1,2,5-oxadiazole attached to position C-28 of lupane frame, displayed selective activity towards MCF7 that was higher than those for betulonic acid **2**: 13.38±5.69 *vs.* 28.78±4.12, respectively, and besides was found less toxic to non-cancer cells. Acylation of **14b** led to acetate **14c**, which, however, was poorly active to all cells tested. Unlike ursane derivative **8b**, the introduction of the 3-O-acetate group in **14c** did not lead to an activity improvement towards MCF-7 cells. In general, furoxan-triazole-containing hybrids of betulinic acid **14b**,**c** showed greater cytotoxicity compared to the parent betulonic derivative **14a**. It should be noted that betulonic acid-derived hybrids with furoxan and amino acid linkers 1**6a**, **b**, **c** [31] exhibited no cytotoxic properties. At the same time, notable anti-inflammatory activity was previously detected for compounds **16 a**, **b**, **c** [31].

The highest activity was found for triple ursane hybrids **8a**, **8b** which demonstrated generally higher cytotoxicity levels than their lupane analogs **14a**, **14b**.

Thus, the combination of heterocyclic fragments of 1,2,3-triazole and 3-(methyl)-4-methyl-1,2,5-oxadiazole-2-oxide attached at position 28 of ursolic acid provides conditions for the cytotoxic activity of the hybrid derivatives.

3.3. Molecular modeling results

Taking into account the hybrid structure of the new compounds, *in silico* screening of their possible interaction with both antitumor targets of lupane and ursane triterpenoids and targets of compounds containing 1,2,3-triazole, 1,3,4- and 1,2,5- oxadiazole was performed (Table 2).

Table 2. Molecular targets used for *in silico* screening of possible interaction with novel hybrid compounds

Entry	Target	PDB ID
1	Mitogen-activated protein kinase 1 (Erk1)	6RQ4
2	Mitogen-activated protein kinase 2 (Erk2)	5NGU
3	Akt1 kinase	3OCB
4	Keap1 Kelch domain	4IQK
5	c-Jun N-terminal kinase (JNK)	2P33
6	ABL2 kinase	2XYN
7	Cyclin-dependent kinase 1	6GU6
8	Cyclin-dependent kinase 2	4KD1
9	Cyclin-dependent kinase 6	5L2T
10	Cyclin-dependent kinase 9	3TN8
11	E3 ubiquitin-protein ligase (Mdm2)	5OAI
12	Serine protease hepsin	5CE1
13	Pim kinase	4ALW
14	TGF-beta receptor type-1 (ALK5)	3GXL
15	Aldo-Keto Reductase 1C3 (AKR1C3)	6GXK
16	Heat shock protein 90-alpha (Hsp90-alpha)	5CF0

17	Indoleamine 2,3-dioxygenase 1 (1DO1)	6AZV
18	Phosphoinositide-dependent kinase 1 (PDK1)	3NAX
19	Vascular endothelial growth factor receptor 1 (VEGFR1)	4ZAU
20	Vascular endothelial growth factor receptor 2 (VEGFR2)	4AG8
21	MEK1 kinase	4LMN
22	Tubulin alpha chain	1SA0
23	Apoptosis regulator Bcl-2	6O0K
24	IkB kinase beta	4KIK
25	BTB domain of Keap1	4CXT

Among these targets, only for the model of Mdm2 ligase (PDB ID 5OAI), results comparable to the corresponding known inhibitor nutlin-3A were obtained. There is evidence of the possible ability of some triterpenoids to inhibit the activity of the Mdm2 protein, which is a negative regulator of the tumor suppressor p53 [47,48].

The estimated binding energy of the new compounds in comparison with the known Mdm2 inhibitors is presented in Table 3. Interestingly, the highest theoretical affinity among the new derivatives was observed for compound **8a**, which was active towards tumor cell lines in *in vitro* experiment (Table 1). Compounds with cytotoxic effect found **9a**, **14a**, including a combination of triazole and furoxan linked to position C-28 of triterpenoid were also on the top of the affinity list. On the other hand, only moderate and low affinity was found for furoxan-triazole hybrid **12** linked to the C-3 position of ursane core and furoxan compounds **16a**, **16c** without triazole spacer (shown low cytotoxicity *in vitro*). Therefore, modeling results are generally in agreement with the results of the *in vitro* test.

Table 3. Docking results of new derivatives at the Mdm2 binding site.

Ligand	**Binding energy*, kcal/mol**
B5K	-9.994
Nutlin-3A	-7.537
8a	-7.490
14a	-6.835
16b	-6.779

9a	-6.578
11c	-6.418
11d	-6.298
12	-6.183
11a	-5.961
15a	-5.949
11b	-5.936
14c	-5.610
1	-5.552
16c	-5.487
14b	-5.458
8b	-5.388
7a	-5.322
9b	-5.276
16a	-5.198
2	-5.156
7d	-4.631
7c	-4.628

* value is not genuine binding energy but estimated docking score.

The estimated binding energy of compound **8a** is comparable to that of a nutlin-3A inhibitor. Since only molecules including combinations of 1,2,3-triazole and furoxan connected to C-28 of triterpenoid core demonstrated cytotoxic effects, we compared the features of non-covalent interactions of new compounds **8a**, **8b**, **9a** in the Mdm2 binding site with known inhibitors nutlin-3A and B5K (Figure 2). The Mdm-2 binding site is characterized by a deep hydrophobic pocket into which the nutlin-3A chlorophenyl substituent and the B5K 6-chloro-indole motif are immersed. The formation of stacking interactions with the π-system of the amino acid residue HIS96 is important for the binding of these inhibitors. Also, for the B5K molecule, a hydrogen bond is formed between the proton at the nitrogen atom of the indole ring and the acceptor oxygen of the amino acid residue LEU54. Apparently, the formation of hydrogen bonds between the carboxyl group and amino acid residues PHE55 and GLN59 through the bridging water molecule 312 is also an important binding feature of this inhibitor. New compounds do not interact with this water molecule; however, molecules **8a** and **8b** can form stacking interactions with the HIS96 π-system. The π system of the furoxan ring is involved in the binding of compound **8a**, and in the case of compound **8b**, stacking is noted for the π-systems of the furoxan and triazole cycles. The bonding feature of these compounds is the formation of hydrogen bonds due to the

strong acceptor nature of the 2-oxide oxygen atom of the furoxan motif. In compounds **8a** and **8b**, this atom can interact with the proton of the hydroxyl group of TYR100, while in compound **9a** it can form a hydrogen bond with a proton bound to the nitrogen of the HIS96 imidazole ring. The oxygen atom inside the furoxan ring of compound **9a** can interact with the proton of the hydroxyl group of TYR100. Such a feature of the interactions of compound **9a** leads to a reversal of its hydrophobic triterpene backbone compared to the configuration of compounds **8a** and **8b**. These compounds exhibit a partial penetration of the methyl substituent of cycle E of the triterpene backbone into the hydrophobic pocket of the binding site, which can promote successful binding by the type of known inhibitors.

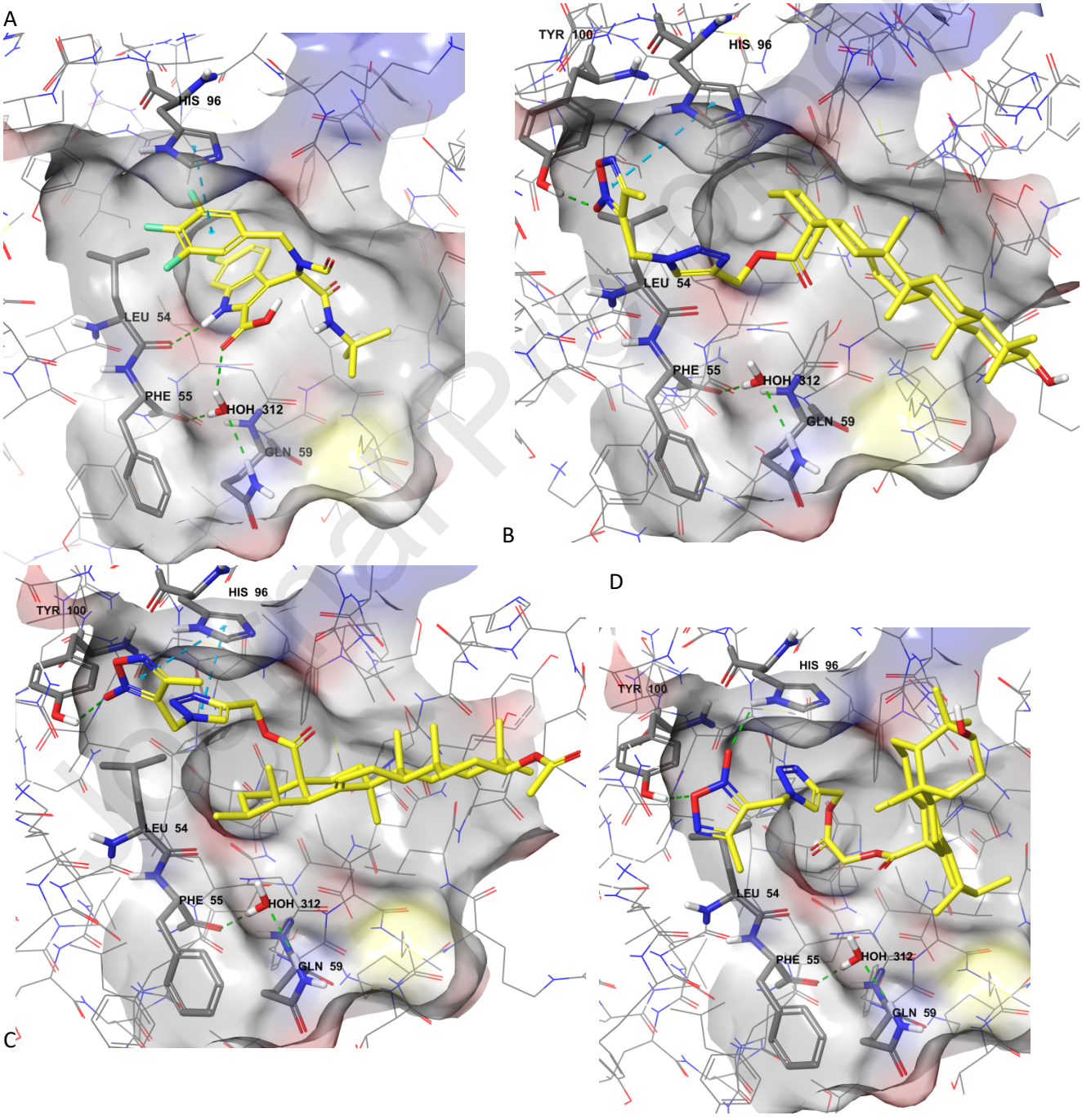


Figure 2. Docking in Mdm2. Non-covalent interactions of compounds (A - B5K, B – **8a**, C – **8b**, D – **9a**) are shown by dashed lines: green - hydrogen bonds, blue - stacking interactions.

3.3. Results of flow cytometry analysis

Mdm-2 oncogene was shown to form a complex with the tumor suppressor p53, and inhibit p53-mediated gene transactivation [49]. Therefore, we studied the impact of nutlin and compounds **8a**, **8b** on the apoptosis and cell cycle of p53-wild and -deficient MCF7 cells. Tables 4, 5 show the data on apoptosis and the cell cycle of MCF7 under the action of a nutlin inhibitor and compounds **8a**, **8b**. From the data presented in table 4 it is seen that **8a**, **8b** do not cause apoptosis in the studied cells within 24 hours. At the same time, when combined with nutlin, both **8a** and **8b** remove the apoptotic effect caused by nutlin. Obviously, compounds 8a, 8b are competitive in the action of nutlin against Mdm2. This may be due to the concurrent penetration of substituents of novel hybrids into the hydrophobic pocket of the binding site of Mdm2 (docking data). The effect of the compounds on the cell cycle is presented in Table 5. The effect of individual compounds, as well as the combined action with nutlin on deficient MCF7 cells, leads to an increase in the number of cells in the S phase (replication stage). The most pronounced effect was noted for **8b** and its combination with nutlin. On deficient cells, one can see a similar character of the action of compound **8b** and nutlin, significantly different from the action of doxorubicin, for which a decrease in the number of cells in the S phase was detected. From the data obtained, it follows that one of the possible mechanisms of the antitumor action of ursolic acid derivatives with a furoxan-triazolo bis-heterocyclic substituent at position C-28 is the binding of compounds to the Mdm2 protein.

Table 4. The effect of nutlin inhibitor and compounds 8a, 8b on apoptosis of MCF-7 a cells [a].

		MCF7 p53 +/+[b]						MCF7 p53 -/-[c]					
		w/o estrogen			with estrogen			w/o estrogen			with estrogen		
Compound	Concentration,uM	normal	apoptosis	necrosis	normal	apoptosis	necrosis	normal	apoptosis	necrosis	normal	apoptosis	necrosis
- control		97.22	1.7	1.08	96.59	0.55	2.86	98.02	0.93	1.05	98.13	0.8	1.07
doxorubicin	2.5	87.22	11.84	0.93	90.14	5.78	4.08	90.04	5.74	4.22	87.67	10.25	2.08
nutlin	17.0	88.29	9.09	2.62	90.85	7.7	1.45	87.31	9.05	3.64	86.62	11.23	2.15
8a	22.9	97.28	0.57	2.15	98.98	0.12	0.9	96.54	0.3	3.16	94.48	0.68	4.84
8a + (nutlin)	22.9+(17.0)	97.79	0.25	1.96	98.84	0.11	1.05	96.84	0.17	2.99	96.18	0.35	3.47
8b	1.55	98.16	0.42	1.42	98.02	0.24	1.74	96.24	0.06	3.7	93.14	0.27	6.59
8b + nutlin	1.55+(17.0)	97.71	0.46	1.83	96.92	0.54	2.54	97.37	0.12	2.51	91.86	1.8	6.34

[a]Data is given as a percentage of cell population; [b] wild type MCF-7; [c] deficient type MCF-7

Table 5. The effect of nutlin inhibitor and compounds 8a, 8b on cell cycle of MCF-7[a].

		MCF7 p53 +/+[b]						MCF7 p53 -/-[c]					
		w/o estrogen			with estrogen			w/o estrogen			with estrogen		
Compound	Concentration,uM	G1	S	G2	G1	S	G2	G1	S	G2	G1	S	G2
- control		65.43	14.14	20.43	61.41	14.35	24.24	57.87	20.46	21.67	66.69	20.86	12.45
doxorubicin	2.5	56.85	5.99	37.16	59.4	6.31	34.29	50.51	4.35	45.14	49.64	8.14	42.22
nutlin	17.0	56.95	16.98	26.07	56.17	18.72	25.11	56.5	24.46	19.04	65.79	22.05	12.16
8a	22.9	69.68	11.89	18.43	64.57	18.09	17.34	63.66	18.43	17.91	64.81	16.85	18.34
8a + (nutlin)	22.9+(17.0)	75.03	12.1	12.87	70.6	13.35	16.05	71.6	13.53	14.87	68.51	14.43	17.06
8b	1.55	66.55	12.11	21.34	56.8	16.89	26.31	47.02	25.73	27.25	61.4	22.83	15.77
8b + (nutlin)	1.55+(17.0)	60.45	16.82	22.73	60.84	17.02	22.14	26.14	32.04	41.82	53.91	25	21.09

[a]Data is given as a percentage of cell population; [b] wild type MCF-7; [c] deficient type MCF-7

4. Conclusion

In conclusion, we designed novel heterocyclic ensembles of 1,3,4- or 1,2,5-oxadiazoles with lupane and ursane triterpenoids tethered with 1,2,3-triazole. Propargyl derivatives of triterpenoids were reacted utilizing click chemistry protocol with 2-azidomethyl-5-aryl-1,3,4-oxadiazoles, and 3-(azidomethyl)-4-methyl-1,2,5-oxadiazole 2-oxide (furoxan derivative). The location of heterocyclic substituents relative to the positions C-3 and C-28 of the triterpenoid frame and ester-type linkers were varied. The obtained new triterpenoid hybrids with 1,2,3-triazoles and 1,3,4- or 1,2,5- oxadiazoles, as well as those including lupane conjugates with aminoacids and 1,2,5-oxadiazoles, were tested for cytotoxic efficacy towards MCF7 breast cancer, A549 lung carcinoma, U-87 MG multiform glioblastoma, and HepG2 hepatocarcinoma cells. The succession of heterocyclic fragments of 1,2,3-triazole and 3-(methyl)-4-methyl-1,2,5-oxadiazole-2-oxide attached at position C-28 of ursane core provided the best cytotoxic activity and selectivity on HepG2 (compound **8a**, activity comparable to doxorubicin) and MCF-7 cells (compound **8b,** activity superior over doxorubicin). The introduction of an additional ester-type linker between triazole and triterpenoid or employing aminoacid-type spacers between the triterpenoid frame and furoxan led to the loss of cytotoxicity. Lupane-type hybrids were found less active compared to their corresponding ursane analogs. According to the molecular docking data supported by flow cytometry analysis data, the most likely mechanism of the cytotoxic action is the affinity of triterpenoid hybrid derivatives to Mdm2 binding sites. The obtained furoxan-triazole-ursane hybrids **8a**, **8b** are candidates for the further mechanism and bioactivity studies.

Acknowledgements

This research was financially supported by the ERA.Net RUS Plus project # RUS_ST2017-139 "AnticancerBet" (Foundation of Basic Research Grant 18-53-76001)). Immortalized human fibroblasts were kindly provided by A. Schilov (Institute of Cytology and Genetics of Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia). Analytical and spectroscopic studies were performed at the Chemical Service collective Center of SB RAS.

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Table 1. Concentrations of half-maximal inhibition (IC_{50} ± SEM, μM) of test compounds on immortalized human fibroblasts, MCF-7, U-87 MG, A549, HepG2 cells.

Compound*	immortalized	MCF7	U-87 MG	A549	HepG2

	human fibroblasts				
Doxorubicin	3.33±0.67	4.51±1.12	2.05±0.22	6.17±1.17	10.02±1.67
Ursolic acid **1**	90.89±5.5	25.05±3.17	43.82±3.88	41.02±3.77	37.28±5.02
Betulonic acid **2**	16.7±2.99	28.78±4.12	45.12±6.78	10.91±1.13	45.0±6.7
8a	25.85±3.04	22.9±10.02	29.71±6.52	>100	12.89±2.63
8b	10.41±1.25	1.55±0.08	>100	>100	>100
9a	82.59±16.87	26.23±8.76	>100	>100	35.58±8.83
11a	74.9±4.58	>100	>100	40.26±7.55	>100
12	48.35±11.47	>100	81.67±2.89	>100	>100
14a	17.48±2.11	>100	>100	>100	54.13±7.05
14b	22.46±2.50	13.38±5.69	>100	>100	>100
14c	33.16±2.77	45.21±6.18	36.33±5.11	41.08±7.06	88.27±9.71

* Compounds **7a**, **7c**, **7d**, **9b**, **11b**, **11c**, **11d**, **11e**, **11f**, **15a**, **16a**, **16b**, **16c** were found non-active (IC_{50} >100 μM for all cells tested)

Table 2. Molecular targets used for *in silico* screening of their possible interaction with novel hybrid compounds

Entry	Target	PDB ID
1	Mitogen-activated protein kinase 1 (Erk1)	6RQ4
2	Mitogen-activated protein kinase 2 (Erk2)	5NGU
3	Akt1 kinase	3OCB
4	Keap1 Kelch domain	4IQK
5	c-Jun N-terminal kinase (JNK)	2P33
6	ABL2 kinase	2XYN
7	Cyclin-dependent kinase 1	6GU6
8	Cyclin-dependent kinase 2	4KD1
9	Cyclin-dependent kinase 6	5L2T
10	Cyclin-dependent kinase 9	3TN8
11	E3 ubiquitin-protein ligase (Mdm2)	5OAI

12	Serine protease hepsin	5CE1
13	Pim kinase	4ALW
14	TGF-beta receptor type-1 (ALK5)	3GXL
15	Aldo-Keto Reductase 1C3 (AKR1C3)	6GXK
16	Heat shock protein 90-alpha (Hsp90-alpha)	5CF0
17	Indoleamine 2,3-dioxygenase 1 (IDO1)	6AZV
18	Phosphoinositide-dependent kinase 1 (PDK1)	3NAX
19	Vascular endothelial growth factor receptor 1 (VEGFR1)	4ZAU
20	Vascular endothelial growth factor receptor 2 (VEGFR2)	4AG8
21	MEK1 kinase	4LMN
22	Tubulin alpha chain	1SA0
23	Apoptosis regulator Bcl-2	6O0K
24	IkB kinase beta	4KIK
25	BTB domain of Keap1	4CXT

Table 3. Docking results of new derivatives at the Mdm2 binding site.

Ligand	**Binding energy*, kcal/mol**
B5K	-9.994
Nutlin-3A	-7.537
8a	-7.490
14a	-6.835
16b	-6.779
9a	-6.578
11c	-6.418
11d	-6.298
12	-6.183
11a	-5.961
15a	-5.949

11b	-5.936
14c	-5.610
1	-5.552
16c	-5.487
14b	-5.458
8b	-5.388
7a	-5.322
9b	-5.276
16a	-5.198
2	-5.156
7d	-4.631
7c	-4.628

* value is not genuine binding energy but estimated docking score.

Table 4. The effect of nutlin inhibitor and compounds 8a, 8b on apoptosis of MCF-7 a cells [a].

		MCF7 p53 +/+[b]						MCF7 p53 -/			
		w/o estrogen			with estrogen			w/o estrogen			
Compound	Concentration,uM	normal	apoptosis	necrosis	normal	apoptosis	necrosis	normal	apoptosis	necrosis	norm
- control		97.22	1.7	1.08	96.59	0.55	2.86	98.02	0.93	1.05	98
doxorubicin	2.5	87.22	11.84	0.93	90.14	5.78	4.08	90.04	5.74	4.22	87
nutlin	17.0	88.29	9.09	2.62	90.85	7.7	1.45	87.31	9.05	3.64	86
8a	22.9	97.28	0.57	2.15	98.98	0.12	0.9	96.54	0.3	3.16	94
8a + (nutlin)	22.9+(17.0)	97.79	0.25	1.96	98.84	0.11	1.05	96.84	0.17	2.99	96
8b	1.55	98.16	0.42	1.42	98.02	0.24	1.74	96.24	0.06	3.7	93
8b + nutlin	1.55+(17.0)	97.71	0.46	1.83	96.92	0.54	2.54	97.37	0.12	2.51	91

[a]Data is given as a percentage of cell population; [b] wild type MCF-7; [c] deficient type MCF-7

Table 5. The effect of nutlin inhibitor and compounds 8a, 8b on cell cycle of MCF-7[a].

		MCF7 p53 +/+[b]						MCF7 p53 -/			
		w/o estrogen			with estrogen			w/o estrogen			
Compound	Concentration,uM	G1	S	G2	G1	S	G2	G1	S	G2	G
- control		65.43	14.14	20.43	61.41	14.35	24.24	57.87	20.46	21.67	66.0
doxorubicin	2.5	56.85	5.99	37.16	59.4	6.31	34.29	50.51	4.35	45.14	49.0
nutlin	17.0	56.95	16.98	26.07	56.17	18.72	25.11	56.5	24.46	19.04	65.7
8a	22.9	69.68	11.89	18.43	64.57	18.09	17.34	63.66	18.43	17.91	64.8
8a + (nutlin)	22.9+(17.0)	75.03	12.1	12.87	70.6	13.35	16.05	71.6	13.53	14.87	68.3
8b	1.55	66.55	12.11	21.34	56.8	16.89	26.31	47.02	25.73	27.25	61.
8b + (nutlin)	1.55+(17.0)	60.45	16.82	22.73	60.84	17.02	22.14	26.14	32.04	41.82	53.9

[a]Data is given as a percentage of cell population; [b] wild type MCF-7; [c] deficient type MCF-7

- Reactions of ursane- and lupane- type alkyns with azole-type azides.
- Triterpenoid hybrids with 1,2,3-triazoles and 1,3,4- or 1,2,5- oxadiazoles.
- MTT test on MCF7, U-87, A549, HepG2 cells.
- The *in silico* evaluation of conjugates affinity to MDM2