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Anticancer effect of A-ring or/and C-ring modified oleanolic acid derivatives on KB, MCF-7 and HeLa cell lines

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New A-ring or/and C-ring modified methyl oleanolate derivatives were prepared. New simple method of synthesis of 3,12-diketone (*3*) from methyl oleanonate (*2*) was worked out. The obtained new compounds were tested for cytotoxic activity on KB, MCF-7 and HeLa cell lines. The derivatives had acetoxy, oxo or hydroxyimino function at the C-3 position and in some cases oxo, hydroxyimino or acyloxyimino group at the C-12 position. Almost all of the compounds showed strong cytotoxic activity, higher than unchanged oleanolic acid. The most active substances turned out to be the derivatives with acyloxyimino function, especially *4* and *8d*.

Despite the progress in technology and medicine, cancer diseases are still the leading cause of death in the world and they pose a great problem for doctors, because the index for cancer cure is still not satisfying and cancer treatment is a challenge. The main reason for this situation is the ability of cancer cells to develop various types of resistance mechanisms, among them the expression of natural or chemically induced multidrug resistance (MDR) and the lack of sensitivity to proapoptotic stimulants. Clinically MDR is characterized by the lack of response to several structurally unrelated drugs acting according to different types of mechanisms. Because of the complexity and versatility of cellular MDR mechanisms, many difficulties are encountered in anticancer drug synthesis and cancer therapy.

As cancer diseases are now one of the most frequent cause of death all over the world, new antitumor agents have been continuously developed. Much attention has been paid to natural products, which have become one of the most important and promising sources of new substances for cytotoxic drug development. It is estimated that about 60% of the antitumor drugs approved for use by regulatory agencies are of natural origin.[1]

Pentacyclic triterpenoids make a class of pharmacologically active and structurally rich natural products with molecules susceptible to further modifications and structure–activity relationship analyses. One of the most popular structures within this group is the oleanane skeleton with oleanolic acid as a representative system. This acid is widely distributed in the plant kingdom, both in the form of free compound and as numerous glycosides. It has been reported to have a broad spectrum of pharmacological activities, including hepatoprotective, antitumor, cardiovascular or immunomodulatory action.[2] Many derivatives of oleanolic acid have been synthesized for a variety of other biological activities.[3–9] For example, it has been reported that amino acid derivatives of oleanolic acid (OA) of different types show the inhibitory activity on the formation of osteoclast-like multi-nucleated cells (OCLs),[3] complement fixation inhibitory[4] and antidiabetic activity.[5] Acylated derivatives of oleanolic acid also turned out to complement fixation inhibitors,[4] act as antidiabetic[5] as well as anti-HIV agents,[6] its esters acted as inhibitors of cancer cell apoptosis[7] and some derivatives with seven-membered lactam system are good transdermal transport promoters.[8] Huand *et al.* have found that oleanonic acid inhibits significantly the growth of cancer cells derived from different tissues.[9]

Some of the above mentioned reports, concerning oleanolic acid derivatives as potential anticancer agents prompted us to embark on an investigation to modify the structure to strengthen the cytotoxic activity. Since the discovery of anticancer activity of oleanolic acid coming from natural sources, interest in synthesis of new compounds based on this triterpene acid, which is easy to obtain, has considerably increased in view of design and production of potential bioactive semi-synthetic compounds. In fact, many authors[10] have reported that modifications of oleanolic acid at the C-3, C-12 and C-28 positions can provide a number of potentially important derivatives with potent anticancer activity.

To explore the effects of the C-3 and C-12 substituents on cytotoxic activity, we converted the C-3 hydroxyl group into acetoxy, oxo and hydroxyimino function or/and the C-12–C-13 double bond within methyl oleanolate molecule to oxo, hydroxyimino or acyloxyimino group.

The starting material for our syntheses was oleanolic acid isolated from by-products formed during the production of ethanolic extract from fresh mistletoe herb (*Viscum alba*). The purity of the obtained triterpene was proven on the basis of spectral data which were in accordance with literature.[11] The spectral data of the product obtained after methylation with dimethyl sulphate were also in agreement with the literature.[12]

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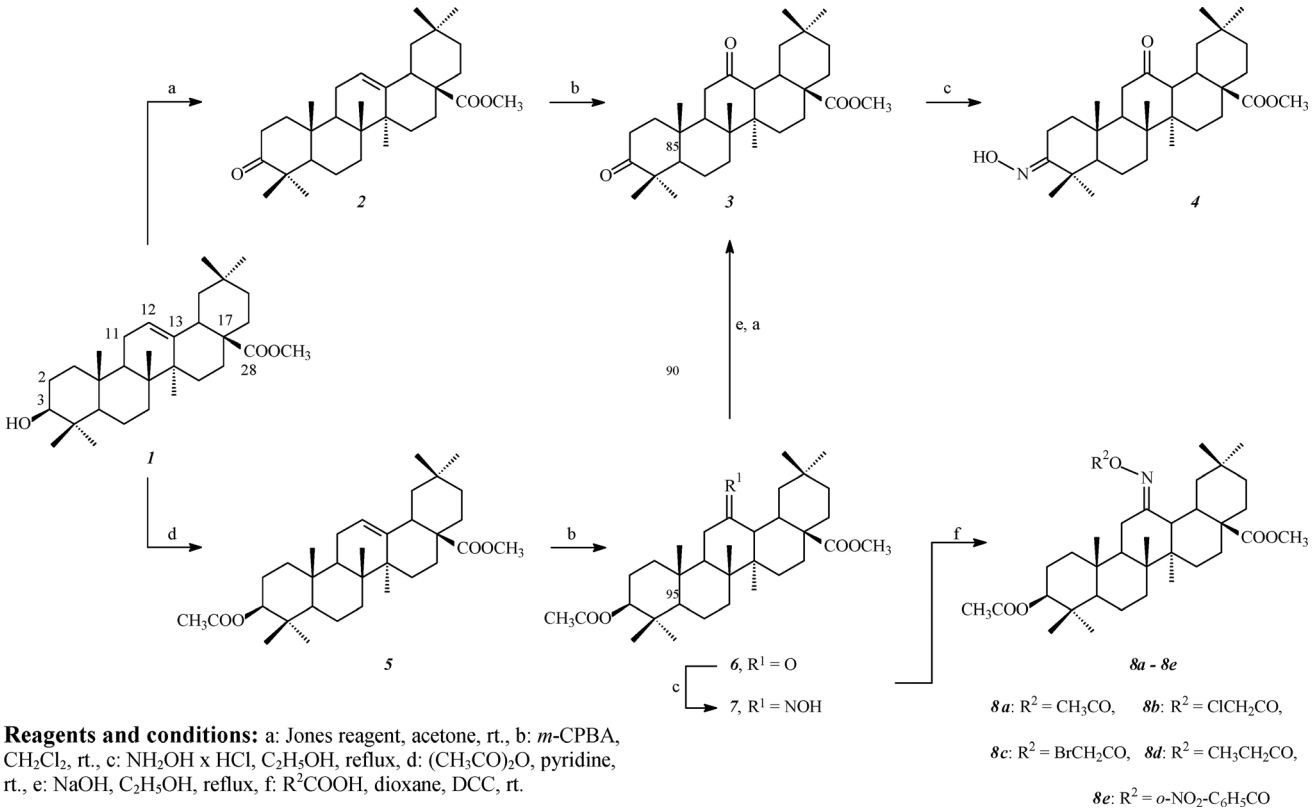

Reagents and conditions: a: Jones reagent, acetone, rt., b: *m*-CPBA, CH_2Cl_2, rt., c: NH_2OH x HCl, C_2H_5OH, reflux, d: $(CH_3CO)_2O$, pyridine, rt., e: NaOH, C_2H_5OH, reflux, f: R^2COOH, dioxane, DCC, rt.

Scheme 1 Synthesis of A- or/and C-ring modified oleanolic acid derivatives.

To obtain new derivatives with A-ring or/and C-ring modifications, the set of transformations presented in Scheme 1 was applied. Some of the characteristic carbon atoms within methyl oleanolate (***1***) in Scheme 1 are numbered. In order to introduce the additional carbonyl group in the C-ring, oleanolic acid was first methylated to its methyl ester (***1***) and the obtained product was then oxidized with Jones' reagent in acetone at room temperature, on the basis of the published procedure.[13] It is known from literature protocol that the C-12–C-13 double bond of acetyl methyl oleanolate can be oxidizied with *m*-CPBA in methylene chloride at room temperature[14] and the compound with C-12 oxo function is obtained as one of the products. It is also known,[15] that the oxidation of C-3 ketoderivative of oleanolic acid ester or other triterpenes with the use of *m*-chloroperbenzoic acid (*m*-CPBA), in the presence of Li_2CO_3, known as Baeyer–Villiger oxidation, leads to a compound with enlarged A-ring containing an additional oxygen atom (A-ring ε-lactone) with the unchanged C-12–C-13 double bond.

Taking under consideration the above mentioned results of oleanolic acid derivatives reactions, the oxidation of methyl 3-oxooleanolate oxidation with *m*-CPBA without lithium carbonate or any other additives was performed.[16]

The obtained mixture of products was subjected to column chromatography. The oxidation of the C-12–C-13 double bond of 3-*O*-acetyl methyl oleanolate (***5***) first led to a compound with C-12–C-13 epoxy function[14] which was subsequently converted to an appropriate 12-oxocompound ***6*** with the use of an iron(III)-picolinate complex.

The application of column chromatography with a column filled with silica gel allowed us to eliminate the necessity of the additional use of chemicals. In this process silica gel acted as an acid catalyst and the opening of the epoxide ring of the intermediate took place. The appropriate 3,12-diketone (***3***) was obtained with a yield of above 80%.

The structure of product ***3***, which was received straight from compound ***2***, with the appliance of the new, simple method, was verified by spectral analysis and the obtained spectral data were in agreement with those from the literature.[17] The obtained 3,12-dioxocompound ***3*** was subjected to the reaction with hydroxylamine hydrochloride in ethanol, on the basis of the procedure known for other oxotriterpenoids.[18] According to the spectral data, the only group that interacted with the above reagent was the 3-oxo function and 3-oxime-12-oxoderivative of methyl oleanolate (***4***), so far unknown in triterpene subject matter, was obtained as the only product.[19]

The second series of derivatives, originated also from methyl oleanolate (***1***), was synthesized following the reaction sequence shown in Scheme 1. Methyl ester of oleanolic acid (***1***) was acetylated with a 10-fold excess of acetic anhydride in anhydrous pyridine[20] to give product ***5***. Then it was oxidized with *m*-CPBA in methylene chloride[14] and after that chromatographed on SiO_2. The deprotection of the hydroxyl group of the resulting 12-ketone (***6***) by boiling the triterpene in 5% ethanolic NaOH gave a 3-hydroxy-12-oxocompound, which was oxidized with Jones' reagent giving only one product: 3,12-dioxoderivative (***3***), identical with that obtained from the earlier transformation

Table 1 The anticancer activity of oleanolic acid derivatives with A-ring or/and C-ring modifications

Comp. no.	IC_{50} values ($\times 10^{-2}$, $\mu M\ L^{-1}$) KB (*carcinoma nasopharynx*)	MCF-7 (breast cancer)	HeLa (cervical cancer)
3	6.19 ± 0.12	>15.00	5.03 ± 0.17
4	1.74 ± 0.04	1.60 ± 0.08	1.80 ± 0.02
6	8.51 ± 0.13	>15.00	7.38 ± 0.13
7	2.06 ± 0.07	11.27 ± 0.09	1.34 ± 0.06
8a	>15.00	>15.00	>15.00
8b	9.42 ± 0.46	7.26 ± 0.33	9.19 ± 029
8c	4.90 ± 0.37	3.76 ± 0.33	4.41 ± 0.36
8d	0.72 ± 0.04	2.13 ± 0.05	1.87 ± 0.09
8e	10.10 ± 0.40	9.28 ± 0.32	9.84 ± 0.82
OA[a]	14.93 ± 0.13	13.95 ± 0.09	11.82 ± 0.04

[a] Reference compound: oleanolic acid (OA)

of ketone ***2***. 12-Oxoderivative ***6*** was also exposed to hydroxylamine hydrochloride in ethanol according to a known method.[18] The spectral data of the obtained product *7*, also so far unknown in triterpene subject matter, confirmed the expected structure of this compound.[21] In the presented NMR spectral data of the all newly obtained compounds only the most characteristic signals are included.

The hydroxy group within hydroxyimino function of compound *7* was acylated with carboxylic acids. The reaction was performed in dioxane in the presence of DCC (dicyclohexylcarbodiimide) at room temperature. Simple aliphatic or aromatic carboxylic acids were applied as acylating agents. After 30–60 min of stirring the acylation was complete as TLC control shown.[22] The spectral data confirmed the expected structure of the products obtained.[23]

The biological tests were performed for two known compounds: diketone ***3*** and 12-monoketone ***6*** as well as for seven new compounds: 3-oxime-12-ketone ***4***, 12-monooxime *7* and for acylated 12-monooximes ***8a–8e***. As known from literature data[24] the introduction of propionoxy group into the molecule of triterpene can improve the pharmacological activity of the new substance, so the compound ***8d***, with propionoxyimino function at the C-12 position was expected to be the most active against the tested cell lines.

The results of these tests were converted to the values summarized in Table 1 with the usage of the formula known from literature protocol.[25]

Unmodified oleanolic acid (OA) was applied as a reference compound with IC_{50} values for KB, MCF-7 and HeLa cell lines of 14.93, 13.95 and 11.82 μM, respectively (see Table 1). The IC_{50} values for the tested oleanolic acid derivatives ***3***, ***4***, ***6***, *7* and ***8a–8e*** ranged from 0.72 to 10.10 μM for KB cells (apart from ***8a***, which was inactive, IC_{50} > 15.00 μM), from 1.60 to 11.27 μM (except for ***3***, ***6*** and ***8a*** with IC_{50} > 15.00 μM) for MCF-7 cells and from 1.34 to 9.84 μM (also apart from ***8a***, which was inactive, IC_{50} > 15.00 μM) for HeLa cells. Analysis of the chemical structures of the tested compounds and their anticancer activity has shown a strong correlation between them. The derivative ***6***, with a 3-*O*-acetyl group and oxo function at the C-12 position was about 1.5-fold more active against KB and HeLa cells (IC_{50} 8.51 and 7.38 μM, respectively) in comparison to oleanolic acid (OA) and inactive against the MCF-7 cell line. The replacement of the 3-acetoxy group in 12-ketone ***6*** with oxo function (diketone ***3***) led to a compound which was about 2.5-fold more active against KB cells (IC_{50} 6.19 μM) and against HeLa (IC_{50} 5.03 μM) cells than OA. The transformation of the C-3 oxo group of product ***3*** into a hydroxyimine function (monooxime ***4***) led to a compound which was suspected to be much more active, as some of the biological tests[26] have shown that compounds having hydroxyimino group within the molecule of triterpene belong to a class of the most active triterpenic species. In fact, the oximation of diketone ***3*** to monooxime ***4*** caused significant intensification of cytotoxic activity against all of the tested cell lines: ketoxime ***4*** was almost 9-fold more active against KB and MCF-7 (IC_{50} 1.74 and 1.60 μM, respectively) and 6.5-fold more active against HeLa cells (IC_{50} 1.80 μM), in comparison to OA. When the ketone function at the C-3 of compound ***3*** was replaced with an acetoxy group and the second ketone group, at C-12, was transformed into a hydroxyimine (product *7*), the new compound turned out to be 7-fold more active against the KB cell line (IC_{50} 2.06 μM) and almost 9-fold more active against the HeLa cell line (IC_{50} 1.34 μM) than the starting material, OA. The introduction of an acetyl group instead of the proton within the C-12 hydroxyimino function (newly obtained compound ***8a***) led to a compound which was inactive against all of the tested cell lines—the IC_{50} value exceeded 15.00 μM. This compound possessed two acetoxy groups within the molecule and it is probable that the 12-acetyloxyimino function weakens the anticancer activity of the obtained compound ***8a***. In the molecules of new products ***8b–8e***, which also had 3-acetoxy function, the 12-acetyloxyimino group was modified (***8b***, ***8c***, ***8e***) or replaced with the 12-propionyloxyimino function (***8d***). The replacement of the 12-acetyloxyimino group with the chloroacetyloxyimino function made the new compound ***8b*** about 1.6-fold more active against KB cells with comparison to mother OA and at the same time the product ***8b*** was about 1.3-fold more active than OA against MCF-7 and HeLa cell lines. The compound ***8c***, with a bromoacetyl function instead of a hydroxyimino proton was about 3–4-fold more active against KB, MCF and HeLa cell lines in comparison to OA. The acylation of the 12-oxime group of compound *7* with simple propionic acid resulted in significant intensification of anticancer activity, especially against KB cells: the propionyloxyimino compound ***8d*** was about 21-fold more active against the above mentioned cell line (IC_{50} 0.72 μM) than OA and about 6.5-fold more active against MCF-7 and HeLa cells (IC_{50} 2.13 and 1.87 μM, respectively). The compound with an *o*-nitrobenzoyl function instead of a hydroxyimino proton (***8e***) turned out to be about 1.5-fold more active against all of the tested cell lines in comparison to the reference compound.

In the biological experiments, KB and HeLa cells were cultured in RPMI 1640 media, MCF-7 cells were cultured in D-MEM media. Both media were supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycine solution. The cell lines were maintained at 37 °C and 5% CO_2 in an incubator. The optimal plating density of cell lines was determined to be 5×10^4 cells.

The protein-staining sulforhodamine B (SRB, Sigma-Aldrich) microculture colorimetric assay, developed by the National Cancer Institute for *in vitro* antitumor screening, was used in this

study to estimate the cell number by providing a sensitive index of total cellular protein content, being linear to cell density. The monolayer cell culture was trypsinized and the cell count was adjusted to 5×10^4 cells. To each well of a 96-well microtiter plate, 0.1 mL of the diluted cell suspension (approximately 10 000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was washed out and 100 μL of six different compound concentrations (0.1 μM, 0.2 μM, 1.0 μM, 2.0 μM, 10.0 μM, 20.0 μM) were added to the cells in microtiter plates. The compounds investigated were dissolved in DMSO (20 μL) and the content of DMSO did not exceed 0.1%, the concentration was found to be nontoxic to the cell lines. The cells were exposed to compounds for 72 h. After this time, 25 μL of 50% trichloroacetic acid was added to the wells and the plates were incubated for 1 h at 4 °C. Then the plates were washed out with the distilled water to remove traces of medium and then dried in air. The air-dried plates were stained with 100 μL SRB and kept for 30 min at room temperature. The unbound dye was removed by rapidly washing with 1% acetic acid and then air dried overnight. The optical density was read at 490 nm.[27] All cytotoxicity experiments were performed three times and the values presented in Table 1 are the mean values. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells).

The cytotoxic activity of semi-synthetic oleanane-type derivatives has been investigated. The overall results suggest that some of the new oleanolates can effectively inhibit the growth of KB, MCF-7 and HeLa cancer cell lines at microgram concentrations and could be promising new anticancer agents. The hydroxyimino derivatives were shown to be more potent anticancer agents in comparison to their mother compounds. The substitution of a hydrogen atom within a hydroxyimino function with propionyl group, led to a further significant intensification of anticancer activity. The introduction of an electron withdrawing substituent into the acyloxyimino group on the C-12 atom led to a moderate increase in anticancer activity.

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16 The synthesis was performed as follows: To a solution of triterpene ***2*** (940 mg, 2.0 mmol) in dried CH_2Cl_2 (15 mL), a solution of a 2-fold amount (690 mg) of *m*-CPBA in dried CH_2Cl_2 (10 mL) was added and the resulting solution was left in darkness at room temperature overnight. After that it was washed successively with 5% solutions of $FeSO_4$, Na_2CO_3, HCl and finally water. The organic solution was dried, filtered, evaporated to dryness and the resulted residue was chromatographed on silica gel. Compound ***3***: yield: 710 mg (73.4%), mp.: 158 °C (C_6H_6), white needles
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19 Compound ***4***: yield: 850 mg (85.2%); mp.: 194–195°C (ethanol), white needles; mol. mass: 499.73; IR (KBr; ν, cm^{-1}): 3410 (OH, N-OH); 1720 (C═O, $COOCH_3$); 1705 (C═O, C-12); 930 (*N*-O, N-OH); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 9.04 (1H, s/br./, N-OH); 3.73 (3H, s, $COOCH_3$); 2.79 (1H, dt, J = 3.4 and 11.6 Hz, C_{18}–H); 2.62 (1H, d, J = 4.1 Hz, C_{13}–H); 1.16; 1.06; 0.99; 0.97; 0.97; 0.92; 0.90 (21H, singlets, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$; δ, ppm): 211.4 (C-12); 178.4 ($COOCH_3$); 166.3 (C-3); 51.8 ($COOCH_3$); 48.1 (C-17); 43.3 (C-13); 32.7 (C-18); DEPT: 8 × CH_3, 10 × CH_2, 4 × CH; EIMS (*m*/*z*): 499.3 (30.9%) $M^{+\cdot}$
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21 For compound ***7***: yield 848 mg (78.0%); mp.: 196–198.5 °C (ethanol), white needles; mol. mass: 543.79; IR (KBr; ν, cm^{-1}): 3430 (OH, N-OH); 1730 and 1710 (C═O, CH_3COO and $COOCH_3$); 905 (*N*-O, N-OH); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 8.05 (1H, s/br/, N-OH); 4.47 (1H, dt, J = 5.7 and 10.5 Hz, C_3-H); 3.67 (3H, s, $COOCH_3$); 2.85 (1H, dt, J = 3.3 and 11.0 Hz; C_{18}-H); 2.52 (1H, d, J = 3.7 Hz; C_{13}-H); 2.04 (3H, s, CH_3COO); 0.91; 0.91; 0.88; 0.87; 0.85; 0.85; 0.81 (7 × 3H, 7 × s, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$; δ, ppm): 178.5 (C-28); 170.8 (CH_3COO); 159.8 (C-12); 80.7 (C-3); 51.8 ($COOCH_3$); 48.1 (C-17); 43.3 (C-13); 32.7 (C-18); 21.3 (CH_3COO); EIMS (*m*/*z*): 543.4 (49.6) $M^{+\cdot}$; Anal. calcd for $C_{33}H_{53}NO_5$ (%): C = 72.89; H = 9.82; N = 2.58; found: C = 72.53; H = 9.86; N = 2.85
22 The synthesis was performed as follows (general method): To a stirred at room temperature solution of compound ***7*** (544 mg, 1.0 mmol) in dioxane (9 mL) 1.2 mmol of carboxylic acid and 1.5 mmol of DCC was added. The stirring was continued at room temperature for about 30 min. The resulting precipitate was filtered, washed with dioxane and the filtrate was poured into a 5-fold volume of water slightly acidified with HCl. The obtained precipitate was filtered, washed with water, dissolved in ethanol and reprecipitated with water. The white solid was filtered, washed with water and dried. Yield >90%
23 For compound ***8a***: yield: 562 mg (95.2%); mp.: 120–125 °C (precipt. with water from ethanolic solution), white powder; mol. mass: 585.82; IR (KBr; ν, cm^{-1}): 1765 (C═O, CH_3COON); 1730 (C═O, $COOCH_3$); 1710 (C═O, CH_3COO); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 4.48 (1H, dd, J = 4.8 and 11.1 Hz, C_3–H); 3.69 (3H, s, $COOCH_3$); 2.69 (1H, d, J = 3.5 Hz, C_{18}–H); 2.25 (3H, CH_3COON); 2.13 (1H, d, J = 13.2 Hz, C_{13}–H); 2.05 (3H, s, CH_3COO); 0.95, 0.93, 0.92, 0.88, 0.86, 0.85 × 2 (21H, singlets, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$; δ, ppm): 178.5 ($COOCH_3$); 171.2 (CH_3COON); 170.8 (CH_3COO); 166.0 (C-12); 80.3 (C-3); 51.8 ($COOCH_3$); 47.9 (C-17); 43.9 (C-13); 32.5 (C-18); 22.2 (CH_3COON); 21.3 (CH_3COO); DEPT: 10 × CH_3, 10 × CH_2, 5 × CH; EIMS (*m*/*z*): 585.5 (0.5%) $M^{+\cdot}$. For compound ***8b***: yield: 600 mg (96.8%); mp.: 118–124 °C (precipt. with water from ethanolic solution), white powder; mol. mass: 620.27; IR (KBr; ν, cm^{-1}): 1750 (C═O, $ClCH_2COON$); 1735 (C═O, $COOCH_3$); 1715 (C═O, CH_3COO); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 4.47 (1H, dd, J = 4.4 and 11.4 Hz, C_3–H); 4.34 (2H, d, J = 2.7 Hz, $ClCH_2COO$); 3.69 (3H, s, $COOCH_3$); 2.73 (1H, d, J = 3.9 Hz, C_{18}–H); 2.05 (3H, s, CH_3COO); 1.94 (1H, d, J = 13.0 Hz, C_{13}–H); 0.96, 0.94, 0.93, 0.88, 0.86, 0.85 × 2 (21H, singlets, 7 × CH_3); ^{13}C NMR (δ, ppm): 178.4 ($COOCH_3$); 170.9 (CH_3COO); 168.2 (C-12); 166.7 ($ClCH_2COON$); 80.3 (C-3); 51.8 ($COOCH_3$); 47.9 (C-17);

44.2 (C-13); 32.5 (C-18); 33.1 ($ClCH_2COON$); 21.3 (CH_3COO); DEPT: 9 × CH_3, 11 × CH_2, 5 × CH; MS (*m*/*z*): 620.7 (3.0%) $M^{+\cdot}$. For compound ***8c***: yield: 610 mg (92.5%); mp.: 110–116 °C (precipt. with water from ethanolic solution), white powder; mol. mass: 664.72; IR (KBr; ν, cm^{-1}): 1740 (C═O, $BrCH_2COON$); 1725 (C═O, $COOCH_3$); 1715 (C═O, CH_3COO); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 4.47 (1H, dd, *J* = 4.7 and 11.4 Hz, C_3–H); 4.08 (2H, d, *J* = 0.6 Hz, $BrCH_2COO$); 3.69 (3H, s, $COOCH_3$); 2.73 (1H, d, *J* = 3.9 Hz, C_{18}–H); 2.3 (1H, dd, *J* = 2.4 and 10.7 Hz, C_{13}–H); 2.05 (3H, s, CH_3COO); 0.96, 0.95, 0.93, 0.88, 0.86, 0.85 × 2 (21H, singlets, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$; δ, ppm): 178.4 ($COOCH_3$); 170.9 (CH_3COO); 168.3 (C-12); 166.2 ($BrCH_2COON$); 80.3 (C-3); 51.8 ($COOCH_3$); 47.9 (C-17); 44.2 (C-13); 32.5 (C-18); 25.5 ($BrCH_2COON$); 21.3 (CH_3COO); DEPT: 9 × CH_3, 11 × CH_2, 5 × CH; EIMS (*m*/*z*): 664.6 (1.2%) $M^{+\cdot}$. For compound ***8d***: yield: 564 mg (94.0%); mp.: 100–105 °C (precipt. with water from ethanolic solution), white powder; mol. mass: 599.85; IR (KBr; ν, cm^{-1}): 1760 (C═O, CH_3CH_2COON); 1730 (C═O, $COOCH_3$); 1710 (C═O, CH_3COO); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 4.47 (1H, dd, *J* = 4.9 and 11.3 Hz, C_3–H); 3.69 (3H, s, $COOCH_3$); 2.79 (1H, d, *J* = 3.8 Hz, C_{18}–H); 2.58–2.51 (2H, m, CH_3CH_2COON); 2.18 (1H, d, *J* = 13.4 Hz, C_{13}–H); 2.05 (3H, s, CH_3COO); 1.20 (3H, s, CH_3CH_2COON); 0.97, 0.94, 0.92, 0.88, 0.86, 0.85 × 2 (21H, singlets, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$; δ, ppm): 178.5 ($COOCH_3$); 173.8 (CH_3CH_2COON); 170.9 (CH_3COO); 166.2 (C-12); 80.3 (C-3); 51.8 ($COOCH_3$); 48.0 (C-17); 44.0 (C-13); 32.5 (C-18); 26.6 (CH_3CH_2COON); 21.3 (CH_3COO); DEPT: 10 × CH_3, 11 × CH_2, 5 × CH; EIMS (*m*/*z*): 599.3 (2.7%) $M^{+\cdot}$. For compound ***8e***: yield: 647 mg (93.8%); mp.: 164–169 °C (precipt. with water from ethanolic solution), white powder; mol. mass: 692.89; IR (KBr; ν, cm^{-1}): 3030 (C-H, Ar); 1750 (C═O, *o*-NO_2-Ar-COON); 1725 (C═O, $COOCH_3$); 1710 (C═O, CH_3COO); 1520 and 1340 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$; δ, ppm): 8.06 (1H, dd, *J* = 1.2 and 7.9 Hz) and 7.97 (1H, dd, *J* = 0.9 and 8.1) and, 7.84–7.62 (2H, m) Ar-H; 4.48 (1H, dd, *J* = 5.1 and 11.5 Hz, C_3–H); 3.70 (3H, s, $COOCH_3$); 2.67 (1H, d, *J* = 3.9 Hz, C_{18}–H); 1.92(1H, dd, *J* = 6.8 and 10.2 Hz, C_{13}–H); 2.05 (3H, s, CH_3COO); 0.92, 0.88, 0.86, 0.85, 0.84 × 2, 0.73 (21H, singlets, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$; δ, ppm): 178.5 ($COOCH_3$); 170.9 (CH_3COO); 169.2 (C-12); 156.7 (*o*-NO_2-Ar-COON); 147.5, 134.3, 133.8, 133.3, 130.8, 123.8 (*o*-NO_2-Ar-COON); 80.4 (C-3); 51.8 ($COOCH_3$); 48.0 (C-17); 44.3 (C-13); 32.4 (C-18); 21.3 (CH_3COO); DEPT: 9 × CH_3, 10 × CH_2, 9 × CH; EIMS (*m*/*z*): 692.8 (0.9%) $M^{+\cdot}$

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