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Synthesis of new fatty acid derivatives of oleanane and ursane triterpenoids and investigation of their *in vitro* cytotoxic effects on 3T3 fibroblast and PC3 prostate cancer cell lines

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Abstract: In this study, 12 new oleanane and ursane derivative triterpene compounds, having fatty acids in the form of esters with carbon numbers of 12, 13, 18, 19, 24 and 25, were synthesized starting from natural products oleanolic and ursolic acids. Initially, 3-methylerythrodiol (3β-methoxyolean-12-en-28-ol) and 3-methyluvaol (3β-methoxyurs-12-en-28-ol) were synthesized from oleanolic acid and ursolic acid, respectively. For this purpose, secondary OH group at C-3 of oleanolic and ursolic acids were protected as methyl ether and, then, their carboxylic acid moieties were reduced by aluminum hydride. New fatty acid derivatives **5a-f** and **8a-f** were synthesized through the reaction of 3-methylerythrodiol/3-methyluvaol and corresponding fatty acid halides. *In vitro* cytotoxic activities of the all synthesized compounds were investigated on 3T3 fibroblast cells and PC3 prostate cancer cell lines. While all the compounds showed at least 70% inhibition on PC3 prostate cancer cells at a concentration of 25 µM, they had average of 50% inhibition on 3T3 fibroblast human healthy cells at the same concentration. Compounds **5c** and **8c** demonstrated the least toxic effect on 3T3 fibroblast human healthy cells and the highest toxic effect on PC3 prostate cancer cells at a concentration of 12.5 µM. Moreover, compounds **8c** and **8e** had the least toxic effect on 3T3 fibroblast human healthy cells and the highest toxic effect on PC3 prostate cancer cells at the same concentration.

Keywords: Oleanolic acid; ursolic acid; triterpenoids; prostate cancer; MTT, cytotoxicity.

1. Introduction

Cancer is a group of diseases characterized by an uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Compared to normal cells, cancer cells damage DNA and do not have a life cycle like normal cells. Instead, the cancerous cells continue to make new cells having the same damaged DNA. Invading other tissues may end up with death unless the spread is

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controlled[1-4]. Even though cancer can affect people from all ages, the risk of being effected by cancer generally increases with age. In 2007, about 13% of all human deaths worldwide, which means 7.9 million, resulted from cancer. There are approximately 75 million people worldwide living with the diagnosis of cancer, and this number is estimated to reach 21 million per year in 2030. In addition, 17 million patients die each year from cancer [5-9].

The cancerous cells can enter bloodstream/lymph vessels and travel to other parts of body, which is called metastasis. Different types of cancers can act very differently in terms of growth rates or their response to various treatments. Some inherited genetic mutations, hormones or immune characteristics can cause cancer. Moreover, permanent DNA damage by external factors such as radiation, toxic chemicals, tobacco, infectious organisms, and an unhealthy diet may cause cancer as well[1-4].

Prostate cancer is the most commonly diagnosed cancer type and the second most common cause of cancer death among men in US. It accounts for about 1 in 4 newly diagnosed cancers each year. Approximately 250,000 new cases of prostate cancer diagnosed in US in 2010. In the same year, 32,000 men were expected to die from prostate cancer[1]. Most common types of cancer in men in Turkey, lung, prostate, colon, bladder and stomach cancers, respectively. However, while women in Turkey in the incidence of cancer in the world average, the rate for males is higher than the world average. In the USA and Europe in the incidence of cancer in both men and women is much higher as compared to Turkey[8, 10]. As there is no enough information about the risk factors and the causes of prostate cancer, it has many unique characteristics compared to other common cancer forms. Age, ethnicity and genetic factors as well as diets, exercise, sexual activity, smoking, and overweight increase the risk. It has been known that depletion of androgens protects men from prostate cancer; on the other hand, increase of androgen level does not cause prostate cancer compared to low level of androgens[10-12].

Triterpenoids have various biological activities as one of the main secondary metabolites of plants. Anti-oxidant, antibacterial, anti-inflammatory, antiviral, cytotoxic, antitumor/anticancer, etc. activities of oleanane, ursane and lupane triterpenoids have already been determined[13-17]. As a result of Topcu and her research group's studies for many years on plants particularly rich in oleanane and ursane triterpenoids, over 50 triterpenoids were isolated and their structures were elucidated. They were determined to have antibacterial, anti-oxidant, cytotoxic and anticholinesterase activities[18-28]. Moreover, the same group also isolated a series of polyhydroxylated oleanane and ursane triterpenoids from some *Salvia* species during recently studies, and determined their strong Nuclear Factor Kappa B (NF-κB) inhibition activity by *in silico* studies and cell culture assays[29-31]. A few triterpenes from the same series were further investigated by NCI (National Cancer Institute-USA) against 60 cell lines *in vitro* for their cytotoxic activity, two of which, 1*β*,2*α*,3*β*,11*α*-tetrahydroxy-urs-12-ene, 3*β*-acetate, and 1*β*,2*α*,3*β*,11*α*-tetrahydroxy-olean-12-ene, 3*β*-acetate, were found to be highly cytotoxic against renal, non-small cell lung and breast cancer cell lines[29].

Many research groups from different countries, including Topçu, have been investigated ursolic acid and oleanolic acid derivatives. These compounds have shown anticancer, antiviral, anticholinesterase and many other activities[14, 16, 29, 32]. In addition to ursane and oleanane derivatives, the cytotoxic, antiviral and even NF-κB inhibition activities of some lupane derivatives, such as betulinic acid and lupeol, were reported and related studies are still continuing intensively[33]. Furthermore, mechanistic studies about inhibition of NF-κB signaling pathway by different terpenes were reported[34, 35].

Today, there is a high requirement for new anticancer drugs because of the side effects of chemotherapeutics. Despite increasing number of anticancer agents, low selectivity, low bioavailability and multiple drug resistance are significant barriers to successful cancer treatment[36]. Thus, discovery of potent and selective anticancer agents is important. The aim of this study is to develop a potent anticancer drug for prostate cancer. Both terpenes and fatty acids are very important chemicals for living organism. A large number of fatty acids are metabolized in living organisms and almost all of them have unique functions. Combination of biologically active natural compounds and biologically active chemical substances are called hybrid molecules. In this study, a series of hybrid molecules was synthesized and their biological activities were investigated.

2. Experimental

2.1. Chemical Material and Apparatus

All solvents, chemicals and other supplies used in the experiments were purchased from Merck, Sigma Aldrich, TCI Chemicals and other suppliers. Although commercially available chemicals and solvents have high purity, purification procedures were performed as described in the literature, when necessary[37-39]. Dichloromethane (Merck, 106049), dimethyl sulfoxide (Sigma-Aldrich, M81802), dimethyl sulfoxide-D6 (Merck, 103591), ethanol (Merck, 100983), ethyl acetate (Merck, 100789), hexane (Merck, 104374), chloroform (Merck, 102445), chloroform-D1 (Merck, 102446), lauroyl chloride (Sigma-Aldrich, 156930), lithium aluminum hydride (Sigma-Aldrich, 686034), methanol (Merck, 106009), methyl iodide (Sigma-Aldrich, 289566), nonadecanoic acid (TCI Chemicals, N0283), oleanolic acid (Carbone Scientific, C-22557), pentacosanoic acid (TCI Chemicals, P0882), cerium (IV) sulfate (Sigma-Aldrich, 359009), silica gel 60-(Gypsum containing- F_{254}) (Merck, 107749), silica gel 60 for column chromatography (70-230 mesh ASTM) (Merck, 107734), silica gel 60 - TLC (20x20 Al plate - F_{254}) (Merck, 105554), silicone oil (ZAG Chemistry, AK350), sodium hydroxide (Merck, 106462), sodium hydride (in 60% mineral oil) (Sigma-Aldrich, 452912), sodium sulfate (Merck, 106649), stearic acid (Merck, 800673), tetrahydrofuran (Merck , 107025), tetracosanoic acid (TCI Chemicals, T0076), thionyl chloride (Sigma-Aldrich, 320536), tridecanoic acid (TCI Chemicals, T0412), ursolic acid (BOC Sciences, B0084-077000).

In general, column chromatography was performed for chromatographic separations and purifications. In column chromatography, silica gel was used as a stationary phase, mixtures of ethyl acetate and hexane were used as mobile phases. Progress of the experiments and the column chromatographies were monitored by thin layer chromatography (TLC), and detection of spots was conducted using UV light, cerium(IV)sulfate solution 10% in sulfuric acid and heating in stove at 100 °C. Nuclear magnetic resonance (NMR) analyses (^{1}H-NMR and ^{13}C-APT NMR) were used for determination of chemical structures. HRMS analyses were performed for determination of molecular weight.

Melting points were determined by Stuart SMP30 melting point apparatus. ^{1}H-NMR and ^{13}C-APT NMR spectra were recorded by Bruker Avance NEO NMR Spectrometer at 500 and 125 MHz, respectively. Coupling constant values were given in Hertz (Hz). Chemical shifts were reported in δ (parts per million) units relative to the internal standard tetramethylsilane (δ = 0.00 ppm) and the peak splits were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), dd (doublet of doublet) and dt (doublet of triplet). HRMS spectra were recorded using ESI technique by Thermo Fischer Scientific Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer.

2.3. Cell Culture

Human prostat adenocarcinoma PC3 and mouse fibroblast 3T3 cell lines were used in this study. PC3 and 3T3 were grown in DMEM/F12 and DMEM, respectively, both supplemented with 10% FBS and 100 U/mL of penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2. After reaching 80% confluency, the cells were detached using 0.25% trypsin-EDTA. For further experiments, cells were resuspended in the growth medium after collection and centrifugation.

2.4. MTT Assay

A MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess cell viability. Briefly, 5x103 cells were seeded into flat-bottom 96-well plate with growth medium. After 24 h incubation, it was treated with increasing doses of samples for 24 hour and the assay was conducted. The absorbance values were recorded at 540 nm using microplate reader. All the experiments were carried out in triplicates, and the results were presented as a mean ± standard deviation.

2.5. Chemistry

*2.5.1. Synthesis of 3-methyloleanolic acid methyl ester **(3)** and 3-methylursolic acid methyl ester **(6)***

A round bottom flask was charged with freshly distilled THF (300 mL) and added NaH (60%, 175 mmol, 7 g, 8 equiv.) under an inert atmosphere. After heating the mixture for 30 minutes, oleanolic acid or ursolic acid (22 mmol, 10 g, 1 equiv.) and MeI (260 mmol, 16 mL, 12 equiv.) were added and refluxed for 2 days, after which excess sodium hydride was carefully hydrolyzed with water (15 mL) in an ice bath. The reaction solvent was removed under reduced pressure and the residue was washed with water (3x300 mL). Organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by column chromatography packed with silica gel eluting with a mixture of ethyl acetate and hexane (1: 9) to obtain the pure compounds **3** (white solid, 9.0 g, 87% yield) and **6** (white solid, 8.5 g, 82% yield).

*Compound **3**:* m.p.: 168 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.28 (1H, t, *J*= 3.4 Hz, olefinic H-12), 3.61 (3H, s, HC-OCH3), 3.34 (3H, s, O=C-OCH_3), 2.85 (1H, dd, *J*= 4.4, 13.8 Hz, H-18), 2.65 (1H, dd, *J*=4.0, 11.6 Hz, H-3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 177.6, 143.1, 121.7, 87.9, 56.8, 55.0, 50.8, 46.9, 46.0, 45.2, 40.9, 40.6, 38.6, 38.0, 37.6, 36.3, 33.1, 32.4, 31.9, 31.7, 30.0, 27.4, 27.0, 25.2, 22.9, 22.7, 22.3, 21.3, 17.5, 16.1, 15.6, 14.6.; HRMS: Molecular Formula: $C_{32}H_{52}O_3$; Exact Mass: 484.39165; Calculated *m/z* [M+1]+: 485.39947; Experimental: *m/z* [M+1]+: 485.39954

*Compound **6**:* m.p.: 165 °C; ^{1}H-NMR (500 MHz, $CDCl_3$), δ ppm: 5.18 (t, *J* = 3.61, Hz, 1H), 3.54 (s, 3H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.72, 4.32 Hz, 1H), 2.16 (d, *J* = 11.07 Hz, 1H), 1.93 (dt, *J* = 13.42, 4.54 Hz, 1H), 1.84 (dd, *J* = 8.86, 3.64 Hz, 2H), 1.83-1.17 (peak groups belong to aliphatic region, 18H), 1.05 (s, 3H), 0.96 (s, 3H), 0.91 (d, *J* = 6.19 Hz, 3H), 0.89 (s, 3H), 0.71 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$), δ ppm: 177.92, 137.99, 125.48, 88.53, 57.42, 55.63, 52.73, 51.34, 47.93, 41.83, 39.37, 38.91, 38.73, 38.53, 38.38, 36.84, 36.50, 32.84, 30.53, 30.19, 28.03, 28.87, 24.09, 23.47, 23.20, 21.89, 21.10, 18.07, 16.94, 16.79, 16.26, 15.30; HRMS: Molecular Formula: $C_{32}H_{52}O_3$; Exact Mass: 484.39165; Calculated *m/z* $[M+1]^+$: 485.39947; Experimental: *m/z* $[M+1]^+$: 485.39990

*2.5.2. Synthesis of 3-methyl erythrodiol **(4)** and 3-methyluvaol **(7)***

Two necked round-bottom flask was charged with freshly distilled tetrahydrofuran (THF). One of the necks was attached to the nitrogen atmosphere and the other neck was fitted with a glass cap. $LiAlH_4$ (165 mmol, 6.25 g, 10 equiv.) was added quickly and the mixture was cooled to -18 ºC in a salt-ice bath. After stirring for 15 minutes, compound **3** or compound **6** (16.5 mmol, 8 g, 1 equiv.) was added under nitrogen atmosphere and the mixture was refluxed for 24 hours. The progress of the reaction was followed by TLC. After 24 h, water (5 mL) was added. The white aluminum complex, which became gel, was separated by vacuum filtration, then the aqueous solvent was removed under reduced pressure. The residue was washed with water (3x300 mL), the organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to obtain the pure compounds **4** (from 3) and **7** (from 6) as white solids (**4**: 6.95 g, 92% yield; **7**: 6.6 g, 88%).

*Compound **4**:* m.p.: 239°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.18 (t, *J* = 3.55 Hz, 1H), 3.53 (d, *J* = 10.90 Hz, 1H), 3.18(d, *J* = 10.90 Hz, 1H), 3.34 (s, 3H), 2.65 (dd, *J* = 11.70, 4.29 Hz, 1H), 0.98 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.77 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 144.30, 122.42, 88.72, 69.63, 57.58, 55.78, 47.63, 46.54, 42.40, 41.75, 39.87, 38.76, 38.57, 36.97, 34.15, 33.26, 32.64, 31.08, 30.99, 28.17, 25.98, 25.59, 23.66, 23.61, 22.09, 22.05, 18.29, 16.79, 16.39, 15.54; HRMS: Molecular Formula: $C_{31}H_{52}O_2$; Exact Mass: 456.39673; Calculated *m/z* $[M+1]^+$: 457.40456; Experimental: *m/z* $[M+1]^+$: 457.40298

*Compound **7**:* m.p.: 217 °C; ^{1}H-NMR (500 MHz, $CDCl_3$), δ ppm: 5.13 (t, *J* = 3.59Hz, 1H), 3.52 (d, *J* = 10.96 Hz, A part of AB system, 1H), 3.35 (s, 3H), 3.18 (d, *J* = 10.96 Hz, B part of AB system, 1H), 2.66 (dd, *J* = 11.72, 4.33 Hz, 1H), 1.90-1.14 (peak groups belong to aliphatic region, 22H), 1.08 (s, 3H),

0.97 (s, 3H), 0.97 (s, 3H), 0.93 (d, *J* = 8.17 Hz, 3H), 0.81 (t, *J* = 5.85 Hz, 3H), 0.76 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$), δ ppm: 138.71, 125.08, 88.67, 69.90, 57.58, 55.72, 54.02, 47.66, 42.02, 40.04, 39.42, 39.35, 38.72, 38.69, 38.00, 36.88, 35.18, 32.83, 30.63, 28.15, 25.99, 23.42, 23.30, 22.07, 21.36, 18.22, 17.38, 16.79, 16.39, 15.69; HRMS: Molecular Formula: $C_{31}H_{52}O_2$; Exact Mass: 456.39673; Calculated *m/z* $[M+1]^+$: 457.40456; Experimental: *m/z* $[M+1]^+$: 457.40363

2.5.3. General Synthesis of Fatty Acid Chlorides

The corresponding fatty acid (4 g, 1 equiv.) was dissolved in chloroform (150 mL) in a round bottom flask. Then, $SOCl_2$ (4 equiv.) was added and the mixture was refluxed for 8 hours. When the reaction was complete, the solvent and excess thionyl chloride were removed under vacuum by heating the mixture. Thus, the acyl halide of the corresponding fatty acid was obtained as pure. According to ^{13}C-APT NMR spectrum of the acyl halides, the carboxylic acid group was completely converted to acyl halides. In NMR the signal of carboxylic acid carbon, resonating at 180 ppm, shifted to 173 ppm in acyl halides. Since the acyl halides used as intermediate products in this study, NMR spectra of only one acyl halides (pentacosanoyl chloride) is given in supplementary materials part.

Pentacosanoyl chloride: ^{1}H NMR (500 MHz, CCl_3) δ 2.81 (t, *J* = 7.3 Hz, 2H), 1.64 (p, *J* = 7.3 Hz, 2H), 1.30-1.14 (m, 42H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.84, 46.10, 30.92, 28.70, 28.66, 28.64, 28.60, 28.59, 28.51, 28.46, 28.36, 28.32, 28.26, 28.14, 28.06, 27.41, 13.11.

*2.5.4. General Syntheses of Triterpene - Fatty Acid Esters **5a-f** and **8a-f***

A round bottom flask was charged with freshly distilled THF (150 mL) and added NaH (60%, (0.2 g, 8.8 mmol, 4 equiv.) under an inert atmosphere. Erythrodiol-3-methyl ether **(4)** or uvaol-3-methyl ether **(7)** (1 g, 2.2 mmol, 1 equiv.) was added and stirred for 30 minutes at reflux temperature. After 30 minutes, the acyl halide (4.4 mmol, 2 equiv.) of the corresponding fatty acid was added and the mixture was refluxed for 24-48 hours. TLC analysis was performed to monitor the progress of the reaction, after which the reaction flask was transferred to an ice bath and water was added dropwise. When the excess sodium hydride was completely hydrolyzed, the reaction solvent was removed under reduced pressure, the residue was washed with water (3X300 mL) and the crude product was extracted with chloroform (3X250 mL). The organic layer was dried over Na_2SO_4 and filtered. Silica gel (10 g) was added to the organic layer and the solvent was removed under reduced pressure. The final mixture was added into a column chromatography, filled with silica gel, and vacuum filtered using hexane as an eluting solvent to remove apolar impurities such as oil. The column chromatography was continued with ethyl acetate and hexane gradient from 1:19 to 1:10 ratio. The collected fractions were analyzed to obtain the corresponding pure products **5a-f** and **8a-f**.

*Compound **5a**:* m.p.: 182 °C; White solid, 1.20 g, 86 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (t, *J* = 3.6 Hz, 1H), 3.94 (d, *J* = 11.0 Hz, 1H), 3.64 (d, *J* = 11.1 Hz, 1H), 3.29 (s, 3H), 2.59 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.24 (t, *J* = 7.3 Hz, 2H), 1.99 (dd, *J* = 13.6, 4.6 Hz, 1H), 1.90 – 1.27 (multiple peak group of aliphatic region, 22H), 1.23-1.20 (m, fatty acid side chain, 20H), 1.09 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.80 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.98, 143.64, 122.91, 88.64, 70.47, 57.54, 55.75, 47.59, 46.26, 42.49, 41.62, 39.79, 38.71, 38.52, 36.93, 35.84, 34.55, 34.02, 33.19, 32.55, 31.95, 31.47, 30.91, 29.66, 29.64, 29.56, 29.37, 29.32, 29.19, 28.12, 25.99, 25.57, 25.18, 23.60, 23.56, 22.72, 22.34, 22.02, 18.22, 16.71, 16.35, 15.49, 14.16; HRMS: Molecular Formula: $C_{43}H_{74}O_3$; Exact Mass: 638.56380; Calculated *m/z* $[M+1]^+$: 639.57162; Experimental: *m/z* $[M+1]^+$: 639.57410

*Compound **5b**:* m.p.: 181°C; White solid, 1.18 g, 82 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20 (t, *J* = 3.7 Hz, 1H), 4.08 (d, *J* = 11.0 Hz, 1H), 3.78 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 3H), 2.66 (dd, *J* = 11.6, 4.3 Hz, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.14 – 1.36 (multiple peak group of aliphatic region, 21H), 1.26 (m, fatty acid side chain, 22H), 1.16 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.13, 143.45, 123.09, 88.64, 71.56, 59.48, 57.56, 55.73, 47.55, 46.15, 42.84, 42.45, 41.90, 41.60, 39.78, 38.71, 38.52, 36.93, 35.88,

33.94, 33.15, 32.53, 31.94, 31.60, 31.48, 30.88, 29.73, 29.68, 29.65, 29.63, 29.29, 29.19, 28.25, 27.48, 26.00, 25.54, 23.92, 22.71, 18.21, 16.72, 16.34, 15.49, 14.14; HRMS: Molecular Formula: $C_{44}H_{76}O_3$; Exact Mass: 652.57945; *m/z* Calculated [M+1]+: 653.58727; Experimental: *m/z* $[M+1]^+$: 653.58984

*Compound **5c**:* m.p.: 194 °C; White solid, 1.40 g, 88 % yield, ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (t, *J* = 3.7 Hz, 1H), 3.94 (d, *J* = 11.0 Hz, 1H), 3.65 (d, *J* = 11.0 Hz, 1H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.99 (dd, *J* = 13.6, 4.8 Hz, 1H), 1.92 – 1.27 (multiple peak group of aliphatic region, 20H), 1.20-1.16 (m, fatty acid side chain, 32H),1.09 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.80 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.99, 143.63, 122.91, 88.65, 70.49, 57.52, 55.75, 47.59, 46.26, 42.49, 41.61, 39.79, 38.71, 38.52, 36.93, 35.84, 34.54, 34.02, 33.18, 32.55, 31.95, 31.46, 30.90, 29.74, 29.72, 29.55, 29.39, 29.32, 29.19, 28.11, 25.98, 25.57, 25.17, 23.60, 23.55, 22.71, 22.34, 22.02, 18.22, 16.71, 16.35, 15.49, 14.15; HRMS: Molecular Formula: $C_{49}H_{86}O_3$; Exact Mass: 722.65770; Calculated *m/z* $[M+1]^+$: 723.66552; Experimental: *m/z* $[M+1]^+$: 723.66785

*Compound **5d**:* m.p.: 192-194 °C; White solid, 1.27 g, 78 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20 (t, *J* = 3.6 Hz, 1H), 4.02 (d, *J* = 11.0 Hz, 1H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 3H), 2.66 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.06 (dd, *J* = 13.6, 4.8 Hz, 1H), 1.98 – 1.35 (multiple peak group of aliphatic region, 20H), 1.29 – 1.22 (m, fatty acid side chain, 34H), 1.16 (s, 3H), 0.98 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.78, 143.61, 122.89, 88.58, 70.42, 60.05, 57.44, 55.76, 47.60, 46.26, 42.49, 41.58, 39.78, 38.69, 38.52, 36.92, 35.83, 34.48, 34.35, 34.03, 33.18, 32.54, 31.96, 31.47, 30.87, 29.75, 29.40, 28.10, 25.97, 25.56, 25.16, 24.98, 23.59, 23.54, 22.71, 22.32, 18.22, 16.68, 16.34, 15.48, 14.14; HRMS: fragments: *m/z*: $[M+1]^+$: 457.40336, 299.25745

*Compound **5e**:* m.p.: 206 °C; White solid, 1.15 g, 65 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (t, *J* = 3.6 Hz, 1H), 3.94 (d, *J* = 11.0 Hz, 1H), 3.64 (d, *J* = 11.0 Hz, 1H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.99 (dd, *J* = 13.6, 4.6 Hz, 1H), 1.91 – 1.26 (multiple peak group of aliphatic region, 22H), 1.20-1.17 (m, fatty acid side chain, 42H), 1.09 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.80 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.93, 143.63, 122.91, 88.63, 70.95, 70.46, 57.52, 55.76, 47.60, 46.26, 42.49, 41.95, 41.61, 39.80, 38.71, 38.53, 36.93, 35.85, 34.53, 34.03, 33.19, 32.56, 31.96, 31.47, 30.90, 29.75, 29.70, 29.65, 29.57, 29.41, 29.33, 29.19, 28.12, 25.99, 25.57, 25.18, 23.60, 23.56, 22.72, 22.34, 22.02, 18.22, 16.71, 16.36, 15.50, 14.16; HRMS: fragments: *m/z*: $[M+1]^+$: 457.40336, 369.35168

*Compound **5f**:* m.p.: 208°C; White solid, 1.20 g, 66 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20 (t, *J* = 3.6 Hz, 1H), 4.01 (d, *J* = 11.0 Hz, 1H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 3H), 2.66 (dd, *J* = 11.6, 4.3 Hz, 1H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.06 (dd, *J* = 13.6, 4.6 Hz, 1H), 1.97-1.35 (multiple peak group of aliphatic region, 22H), 1.35 – 1.21 (m, fatty acid side chain, 44 H), 1.16 (s, 3H), 0.98 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.89 (s, 6H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 173.95, 143.61, 122.81, 88.67, 70.91, 70.45, 57.50, 55.70, 47.61, 46.20, 42.45, 41.91, 41.65, 39.82, 38.77, 38.55, 36.92, 35.87, 34.57, 34.04, 33.18, 32.57, 31.98, 31.48, 30.92, 29.75, 29.70, 29.65, 29.57, 29.41, 29.33, 29.19, 28.12, 25.99, 25.57, 25.18, 23.61, 23.52, 22.71, 22.37, 22.02, 18.22, 16.76, 16.36, 15.50, 14.19; HRMS: Molecular Formula: $C_{56}H_{100}O_3$; Exact Mass: 820.76725; Calculated *m/z* $[M+1]^+$: 821.77507; Experimental: *m/z* $[M+1]^+$: 821.77257

*Compound **8a**:* m.p.: 185°C; White solid, 1.10 g, 79 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.14 (t, *J* = 3.7 Hz, 1H), 4.06 (d, *J* = 11.0 Hz, 1H), 3.62 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 3H), 2.66 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.04 – 1.33 (multiple peak group of aliphatic region, 23H), 1.28 – 1.25 (m, yağ asiti yan zinciri, 18H), 1.10 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.82 (d, *J* = 4.9 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.87, 138.15, 125.65, 88.63, 70.92, 57.49, 55.75, 54.34, 47.68, 41.93, 39.99, 39.36, 39.24, 38.69, 36.91, 36.87, 35.79, 34.48, 32.80, 31.95, 30.56, 29.67, 29.65, 29.58, 29.38, 29.33, 29.18, 28.14, 26.03, 25.18, 23.44, 23.35,

22.72, 22.05, 21.30, 18.19, 17.32, 16.73, 16.38, 15.65, 14.15; HRMS: Molecular Formula: $C_{43}H_{74}O_3$; Exact Mass: 638.56380; Calculated *m/z* $[M+1]^+$: 639.57162; Experimental: *m/z* $[M+1]^+$: 639.57086

Compound ***8b****:* m.p.: 184 °C; White solid, 1.16 g, 82 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.14 (t, *J* = 3.7 Hz, 1H), 4.06 (d, *J* = 11.0 Hz, 1H), 3.62 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 3H), 2.66 (dd, *J* = 11.6, 4.3 Hz, 1H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.02 – 1.34 (multiple peak group of aliphatic region, 23H), 1.32 – 1.22 (m, fatty acid side chain, 20H), 1.10 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.82 (d, *J* = 5.0 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.75, 138.15, 125.63, 88.58, 70.88, 57.43, 55.75, 54.35, 47.68, 41.92, 39.98, 39.97, 39.36, 39.23, 38.71, 38.67, 36.90, 36.85, 35.79, 34.44, 32.79, 31.95, 31.93, 30.56, 29.72, 29.51, 29.48, 28.13, 26.03, 25.16, 23.44, 23.42, 23.34, 22.71, 22.02, 21.29, 18.19, 17.31, 16.71, 16.37, 15.64, 14.14; HRMS: Molecular Formula: $C_{44}H_{76}O_3$; Exact Mass: 652.57945; Calculated *m/z* $[M+1]^+$: 653.58727; Experimental: *m/z* $[M+1]^+$: 653.58563

Compound ***8c****:* m.p.: 196°C; White solid, 1.48 g, 93 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.07 (t, *J* = 3.7 Hz, 1H), 3.99 (d, *J* = 11.0 Hz, 1H), 3.55 (d, *J* = 11.0 Hz, 1H), 3.28 (s, 3H), 2.59 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.21 (t, *J* = 7.5 Hz, 2H), 1.93 – 1.26 (multiple peak group of aliphatic region, 24H), 1.22 – 1.15 (m, fatty acid side chain, 30H), 1.03 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.81 (t, *J* = 13.3 Hz, 3H), 0.75 (d, *J* = 5.3 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.73, 138.14, 125.62, 88.57, 70.88, 57.41, 55.76, 54.35, 47.68, 41.91, 39.98, 39.36, 39.23, 38.70, 38.67, 36.90, 36.85, 35.79, 34.43, 32.79, 31.95, 30.56, 29.76, 29.73, 29.71, 29.65, 29.58, 29.40, 29.17, 28.12, 26.02, 25.16, 23.43, 23.34, 22.71, 22.02, 21.28, 18.19, 17.30, 16.71, 16.36, 15.64, 14.14; HRMS: Molecular Formula: $C_{49}H_{86}O_3$; Exact Mass: 722.65770; Calculated *m/z* $[M+1]^+$: 723.66552; Experimental: $[M+1]^+$: 723.66786

Compound ***8d****:* m.p.: 197°C; White solid, 1.33 g, 82 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.14 (d, *J* = 3.7 Hz, 1H), 4.06 (d, *J* = 11.0 Hz, 1H), 3.62 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 3H), 2.66 (dd, *J* = 11.6, 4.3 Hz, 1H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.01 – 1.34 (multiple peak group of aliphatic region, 23H), 1.28 – 1.25 (m, fatty acid side chain, 32H), 1.10 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.82 (d, *J* = 4.8 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.87, 138.15, 125.65, 88.63, 70.93, 57.46, 55.76, 54.35, 47.69, 41.94, 40.00, 39.37, 39.24, 38.69, 36.92, 36.87, 35.79, 34.47, 32.80, 31.96, 30.56, 29.75, 29.70, 29.58, 29.40, 29.33, 29.18, 28.13, 26.03, 25.17, 23.43, 23.35, 22.71, 22.04, 21.29, 18.19, 17.31, 16.73, 16.37, 15.65, 14.14; HRMS: fragments: *m/z*: $[M+1]^+$: 457.40336, 299.29398

Compound ***8e****:* m.p.: 208°C; White solid, 1.24 g, 70 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (t, *J* = 3.7 Hz, 1H), 4.01 (d, *J* = 11.0 Hz, 1H), 3.58 (d, *J* = 11.1 Hz, 1H), 3.31 (s, 3H), 2.62 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.98 – 1.31 (multiple peak group of aliphatic region, 23H), 1.28 – 1.11 (m, fatty acid side chain, 42H), 1.05 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.84 (t, *J* = 6.9 Hz, 3H), 0.77 (d, *J* = 5.4 Hz, 3H), 0.73 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.96, 138.15, 125.67, 88.68, 70.96, 57.52, 55.77, 54.35, 47.69, 41.95, 40.01, 39.37, 39.25, 38.71, 36.93, 36.88, 35.79, 34.51, 32.81, 31.96, 30.57, 29.75, 29.71, 29.66, 29.59, 29.41, 29.34, 29.19, 28.15, 26.04, 25.19, 23.45, 23.36, 22.73, 22.07, 21.31, 18.20, 17.33, 16.75, 16.39, 15.67, 14.16; HRMS: fragments: *m/z:* $[M+1]^+$: 457.40336, 369.37305

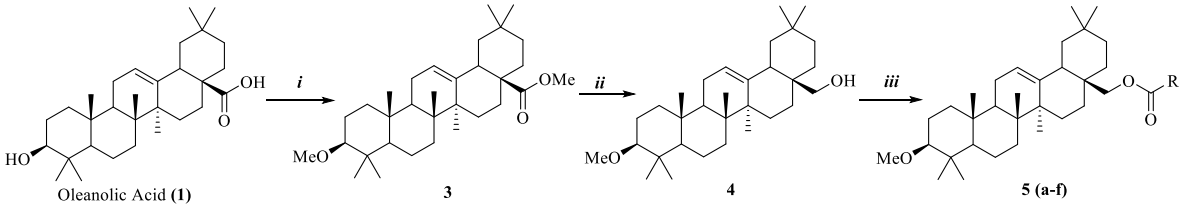
Compound ***8f****:* m.p.: 209 °C; White solid, 1.3 g, 72 % yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.07 (t, *J* = 3.6 Hz, 1H), 3.98 (d, *J* = 11.0 Hz, 1H), 3.56 (d, *J* = 11.0 Hz, 1H), 3.29 (s, 3H), 2.60 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.22 (t, *J* = 7.4 Hz, 2H), 1.96 – 1.28 (multiple peak group of aliphatic region, 23H), 1.18 (s, fatty acid side chain, 44H), 1.03 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.87 (d, *J* = 4.8 Hz, 3H), 0.81 (s, 3H), 0.81 (t, *J* = 6.9 Hz, 3H), 0.75 (d, *J* = 5.2 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.03, 138.15, 125.68, 88.70, 70.97, 69.95, 57.57, 55.76, 54.34, 47.68, 44.18, 41.97, 40.01, 39.37, 39.25, 38.72, 36.93, 36.89, 35.79, 34.54, 32.81, 31.95, 30.56, 29.73, 29.69, 29.66, 29.58, 29.39, 29.33, 29.19, 28.98, 28.92, 28.60, 28.15, 26.47, 26.04, 25.72, 25.19, 23.46, 23.36, 22.72, 22.08, 21.31, 18.20,

17.33, 16.76, 16.39, 15.67, 14.15; HRMS: Molecular Formula: $C_{56}H_{100}O_3$; Exact Mass: 820.76725; Calculated *m/z* $[M+1]^+$: 821.77507; Experimental: *m/z* $[M+1]^+$: 821.77167

3. Results and Discussion

3.1. Syntheses

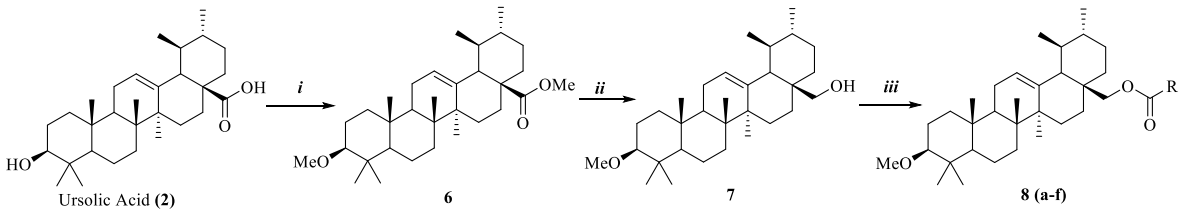
In this study, 12 new oleanane and ursane derivative triterpene compounds, having fatty acids in the form of esters with carbon numbers of 12, 13, 18, 19, 24 and 25, were synthesized starting from natural products oleanolic and ursolic acids. Initially, 3-methylerythrodiol(3β-methoxyolean-12-en-28-ol) **(3)** and 3-methyluvaol (3β-methoxyurs-12-en-28-ol) **(6)** were synthesized from oleanolic and ursolic acids, respectively (Schemes 1 and 2). For this purpose, secondary OH at C-3 and the acid groups of oleanolic and ursolic acids were protected as methyl ethers and esters (**3** and **6**) then, the ester moieties were reduced with aluminum hydride to produce **4** and **7**. New triterpene-fatty acid derivatives **5a-f** and **8a-f** were synthesized from reactions of 3-methylerythrodiol and 3-methyluvaol with corresponding fatty acid halides.



5a: R = $C_{11}H_{23}$ - Laurat derivative, 86%
5b: R = $C_{12}H_{25}$ - Tridecylat derivative, 82%
5c: R = $C_{17}H_{35}$ - Stearat derivative, 88%
5d: R = $C_{18}H_{37}$ - Nonadecanoat derivative, 78%
5e: R = $C_{23}H_{47}$ - Lignocerat derivative, 65%
5f: R = $C_{24}H_{49}$ - Pentacosanoat derivative, 66%

Reagents and conditions:
i) NaH, MeI, THF, N_2, 48h, Reflux, 87%; ***ii)*** $LiAlH_4$, THF, N_2, 24h, Reflux, 88%;
iii) NaH, Corresponding Fatty Acid Halide, THF, N_2, 24-48h, Reflux

Scheme 1. Syntheses of oleanane triterpenoids having fatty acid esters



8a: R = $C_{11}H_{23}$ - Laurat derivative, 79%
8b: R = $C_{12}H_{25}$ - Tridecylat derivative, 82%
8c: R = $C_{17}H_{35}$ - Stearat derivative, 93%
8d: R = $C_{18}H_{37}$ - Nonadecanoat derivative, 82%
8e: R = $C_{23}H_{47}$ - Lignocerat derivative, 70%
8f: R = $C_{24}H_{49}$ - Pentacosanoat derivative, 72%

Reagents and conditions:
i) NaH, MeI, THF, N_2, 48h, Reflux, 87%; ***ii)*** $LiAlH_4$, THF, N_2, 24h, Reflux, 88%;
iii) NaH, Corresponding Fatty Acid Halide, THF, N_2, 24-48h, Reflux

Scheme 2. Syntheses of ursane triterpenoids having fatty acid esters

In the NMR analysis of the compounds, the chemical shift values of the hydrogens and carbons belong to the C-28 position are given comparatively in Table 1 for ursane and oleanane derivatives. The chemical shift values differed ± 0.1 ppm depending on the length of the fatty acid. Due to the increasing carbon number, a marked decrease in the polarities of the compounds were observed. For this reason, the most suitable solvent for all the compounds were found to be chloroform and THF.

Table 1. Chemical shift values of carbons and hydrogens at the 28 position of the synthesized compounds

Oleanane derivatives				Ursane derivatives			
Comp.	**^{1}H-NMR δ ppm**		**^{13}C-NMR δ ppm**	**Comp.**	**^{1}H-NMR δ ppm**		**^{13}C-NMR δ ppm**
4	3.53	3.18	69.63	**7**	3.52	3.35	69.90
5a	3.94	3.64	70.47	**8a**	4.06	3.62	70.92
5b	4.02	3.72	71.56	**8b**	4.06	3.62	70.88
5c	3.94	3.65	70.49	**8c**	3.99	3.55	70.88
5d	4.02	3.71	70.42	**8d**	4.06	3.62	70.93
5e	3.94	3.64	70.95	**8e**	4.01	3.58	70.96
5f	4.01	3.71	70.96	**8f**	3.98	3.56	70.97

3.2. Biological Activity Studies

All the synthesized compounds were investigated for their cytotoxic effects against 3T3 fibroblast human healthy and PC3 prostate cancer cell lines. The cytotoxic effects and IC_{50} values of the compounds on 3T3 fibroblast human health cells are given in Table 2 and the cytotoxic effects and IC_{50} values on PC3 prostate cancer cells are given in Table 3. The graphics of the results in Tables 2 and 3 are given in Figures 1 and 2, respectively.

Table 2. Cytotoxic effects of compounds on 3T3 fibroblast human healthy cells

Concentration	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	3.125 µM	IC_{50} µM
Control	100	100	100	100	100	100	
1	25.1232	57.0334	76.7378	97.0991	130.5966	117.4603	75.74 ± 0.2283
2	39.2308	76.4103	79.4872	91.0256	81.0256	86.4103	89.81 ± 0.1072
3	49.1516	67.5424	89.8741	118.7739	149.6442	135.1943	64.30 ± 0.4286
4	21.8391	31.0345	46.7980	65.8457	83.5796	94.0887	25.05 ± 0.0322
6	61.0256	71.0256	76.4103	82.5641	108.7179	131.7949	135.40 ± 0.2145
7	23.0769	35.3846	36.9231	39.2308	78.4615	100.0000	17.38 ± 0.1149
5a	17.2932	24.3108	24.8120	32.3308	31.3283	32.8321	3.11 ± 0.1700
5b	21.3033	32.8321	36.8421	62.4060	82.9574	61.4035	18.16 ± 0.1137
5c	23.1527	46.1412	70.4434	106.5681	125.6158	151.8883	68.88 ± 0.3337
5d	33.5692	41.8121	48.8081	53.3343	68.2011	75.6610	22.52 ± 0.0912
5e	23.8095	54.1353	68.6717	92.2306	114.7870	91.2281	58.89 ± 0.1322
5f	42.1053	48.1203	51.6291	54.1353	58.1454	58.6466	21.59 ± 0.1930
8a	10.8861	20.5063	25.0633	36.2025	51.8987	64.5570	7.26 ± 0.0470
8b	14.4304	22.5317	22.0253	32.1519	79.7468	80.2532	10.87 ± 0.1016
8c	30.0000	40.7692	46.1539	56.1539	75.3846	83.0769	23.17 ± 0.0755
8d	22.5769	37.1846	38.0231	40.2908	68.9615	80.5400	11.033 ± 0.2567
8e	14.9367	47.9325	63.6287	71.2236	108.6920	105.6540	42.16 ± 0.1283
8f	38.8186	49.9578	54.5148	64.1350	72.7426	90.9705	35.75 ± 0.0934

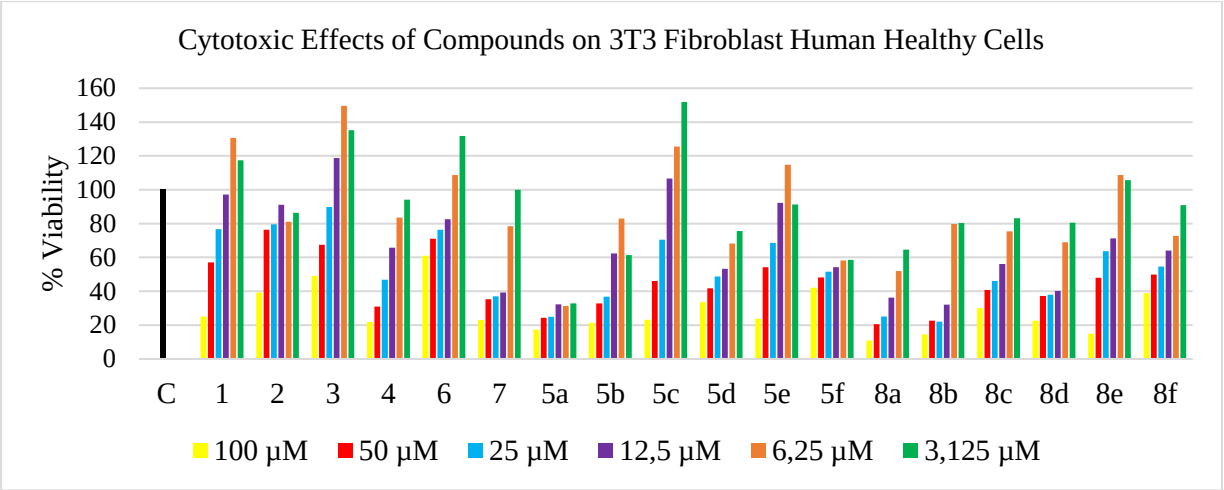


Figure 1. Cytotoxic effects of compounds on 3T3 fibroblast human healthy cells (C: Control)

Table 3. Cytotoxic effects of compounds on PC3 prostate cancer cells

Concentration	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	3.125 µM	IC50 µM
Control	100	100	100	100	100	100	
Cisplatin							399.35
1	5.5493	18.3843	50.6606	67.6482	109.5508	108.0785	26.40 ± 0.1697
2	7.7018	13.0212	13.2676	13.7605	18.3200	35.3255	1.77 ± 0.0851
3	20.9891	19.8566	59.9472	57.5689	69.0072	59.3809	17.39 ± 0.1369
4	5.3228	11.2118	38.8449	53.0011	83.3522	87.5425	15.14 ± 0.0817
6	10.9263	12.4050	14.7464	24.7279	57.1370	118.8745	9.52 ± 0.2060
7	4.7443	6.0998	6.3463	16.0813	32.8404	66.3586	3.692 ± 0.0762
5a	48.6866	36.2408	7.6766	16.6539	35.2206	48.6866	3.712 ± 0.2616
5b	4.9222	7.5746	13.7975	53.4813	77.7608	132.5427	14.63 ± 0.2401
5c	15.6285	11.0985	28.6523	43.6014	58.7769	59.6829	8.26 ± 0.0703
5d	4.5202	7.5998	20.9180	52.4813	76.7208	86.5325	21.93 ± 0.046
5e	4.8202	14.1971	20.3180	51.5345	89.0759	119.3743	15.90 ± 0.1879
5f	34.0899	29.9073	23.2764	25.2147	45.1075	61.3279	6.08 ± 0.1562
8a	3.5081	7.4141	15.2260	47.4864	75.5515	122.5678	13.07 ± 0.2055
8b	3.6528	5.2441	11.0307	32.4412	59.0597	105.0633	8.62 ± 0.1575
8c	5.4837	7.2089	11.8916	28.5274	65.2495	85.3358	7.75 ± 0.0995
8d	11.8263	14.4940	24.3364	44.9239	79.5370	98.4745	26.88 ± 0.0312
8e	4.0868	13.7673	25.6299	60.3496	85.2321	131.6697	18.27 ± 0.2183
8f	4.0000	14.3460	24.4726	52.3930	76.2628	131.5250	15.89 ± 0.2168

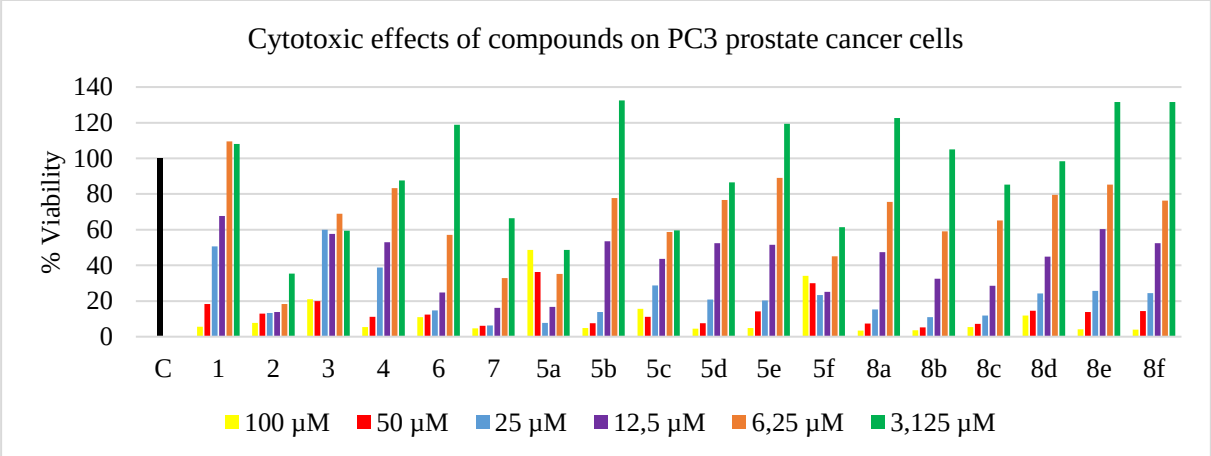


Figure 2. Cytotoxic effects of the compounds on PC3 prostate cancer cells

According to the results of the biological activities, the compounds exhibited cytotoxic effects on 3T3 fibroblast human healthy cells on an average of 2/3 of the cells at a concentration of 100 µM,

and the cytotoxic effects decreased with decreasing doses. Compound **8a** was observed to be the most toxic one against 3T3 fibroblast human healthy cells at a concentration of 100 µM, while the compound with the least toxic effect on 3T3 fibroblast human healthy cells was determined to be **5f.** It was observed that **5c, 5e** and **8e** proliferated 3T3 fibroblast human healthy cells at lower concentrations. The compound with the lowest toxicity at a concentration of 25 µM on 3T3 fibroblast human healthy cells was **5c**.

According to the results of the cytotoxic effects of the compounds on PC3 prostate cancer cells, compound **5a** was the most toxic at a concentration of 3.125 µM. Compound **5a** killed approximately 52% of cancerous cells at a concentration of 3.125 µM, while the effects of other triterpene derivatives appeared to be weaker at this concentration. For TD50, the lowest average concentration that killed at least 50% of cancerous cells was 12.5 µM. These results revealed that the compounds had toxic effects on at least 50% of cancer cells at a concentration of 12.5 µM. At a concentration of 25 µM, all the compounds showed at least 70% toxicity on PC3 prostate cancer cells. These results added a great deal of value to the results of the experiments on healthy cells as the compounds showed less than 50% toxicity on healthy cells at concentrations between 12-25 µM.

4. Conclusions

As a result, the compounds with the least toxic effect on 3T3 fibroblast human healthy cells at a concentration of 12.5 µM and the compounds with the highest toxic effect on PC3 prostate cancer cells at the same concentration were **5c, 5f** and **8c**. At 25 µM concentration, the compounds with the least toxic effect on 3T3 fibroblast human healthy cells and the compounds with the highest toxic effect on PC3 prostate cancer cells at the same concentration were **5c, 5e, 5f, 8e** and **8f.**

Considering the structures of the compounds, **5c** and **8c**, which are more active than the others, have 18 carbons in the side chain (stearic acid ester), while **5e** and **8e** have 24 carbons (tetracosanoic acid ester), which means the compounds having two carbon side chains were more effective than the compounds with one carbon side chain. Thus, the most effective compound was found to have stearic acid side chain.

The compounds, synthesized in this study, showed strong toxicity toward PC3 prostate cancer cells, even at low concentrations, and demonstrated relatively low toxicity on healthy cells. Such a result is very important for the discovery of new chemotherapeutic drugs.

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Supporting Information

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References

[1] Anonymous, Acs releases 2005 edition of cancer facts & figures. *Am. Fam. Physician* **2005,** *71 (8),* 1612-1612.

[2] Hausman, D. M., What is cancer? *Perspect. Biol. Med.* **2019,** *62 (4),* 778-784.
[3] Nygren, P.; Grp, S. B. U., What is cancer chemotherapy? *Acta Oncol.* **2001,** *40 (2-3),* 166-174.
[4] Schram, F. R.; Ng, P. K. L., What is cancer? *J. Crust. Biol.* **2012,** *32 (4),* 665-672.
[5] Popat, K.; McQueen, K.; Feeley, T. W., The global burden of cancer. *Best Pract. Res. Clin. Anaesthesiol.* **2013,** *27 (4),* 399-408.
[6] Parkin, D. M.; Bray, F.; Ferlay, J.; Pisani, P., Global cancer statistics, 2002. *CA Cancer J. Clin.* **2005,** *55 (2),* 74-108.
[7] Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D., Global cancer statistics. *CA Cancer J. Clin.* **2011,** *61 (2),* 69-90.
[8] Chuang, K. Y.; Chuang, Y. C.; Ho, Y. S., Global influence of cancer statistics articles. *Curr. Sci.* **2015,** *109 (9),* 1552-1554.
[9] Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, A., Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2018,** *68 (6),* 394-424.
[10] Zorlu, F.; Divrik, R. T.; Eser, S.; Yorukoglu, K., Prostate cancer incidence in Turkey: An epidemiological study. *Asian Pac. J. Cancer Prev.* **2014,** *15 (21),* 9125-9130.
[11] Attard, G.; Parker, C.; Eeles, R. A.; Schroder, F.; Tomlins, S. A.; Tannock, I.; Drake, C. G.; de Bono, J. S., Prostate cancer. *Lancet* **2016,** *387 (10013),* 70-82.
[12] Ilic, D.; Dahm, P., Prostate cancer screening in europe. *Lancet* **2015,** *385 (9977),* 1506-1506.
[13] Dzubak, P.; Hajduch, M.; Vydra, D.; Hustova, A.; Kvasnica, M.; Biedermann, D.; Markova, L.; Urban, M.; Sarek, J., Pharmacological activities of natural triterpenoids and their therapeutic implications. *Nat. Prod. Rep.* **2006,** *23 (3),* 394-411.
[14] Zhang, Q.; Chang, Z.; Wang, Q., Ursane triterpenoids inhibit atherosclerosis and xanthoma in ldl receptor knockout mice. *Cardiovasc. Drugs Ther.* **2006,** *20 (5),* 349-357.
[15] Ukiya, M.; Akihisa, T.; Tokuda, H.; Suzuki, H.; Mukainaka, T.; Ichiishi, E.; Yasukawa, K.; Kasahara, Y.; Nishino, H., Constituents of compositae plants III. Anti-tumor promoting effects and cytotoxic activity against human cancer cell lines of triterpene diols and triols from edible chrysanthemum flowers. *Cancer Lett.* **2002,** *177 (1),* 7-12.
[16] Topcu, G., Bioactive triterpenoids from salvia species. *J. Nat. Prod.* **2006,** *69 (3),* 482-487.
[17] Liby, K. T.; Yore, M. M.; Sporn, M. B., Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. *Nat. Rev. Cancer* **2007,** *7 (5),* 357-369.
[18] Topcu, G.; Eris, C.; Ulubelen, A.; Krawiec, M.; Watson, W. H., New rearranged neoclerodane diterpenoids from *Teucrium alyssifolium. Tetrahedron* **1995,** *51 (43),* 11793-11800.
[19] Topcu, G.; Tan, N.; Ulubelen, A.; Sun, D.; Watson, W. H., Terpenoids and flavonoids from the aerial parts of *Salvia candidissima. Phytochemistry* **1995,** *40 (2),* 501-504.
[20] Topcu, G.; Ayral, M. N.; Aydin, A.; Goren, A. C.; Chai, H. B.; Pezzuto, J. M., Triterpenoids of the roots of *Lavandula stoechas* ssp stoechas. *Pharmazie* **2001,** *56 (11),* 892-895.
[21] Ertas, A.; Ozturk, M.; Boga, M.; Topcu, G., Antioxidant and anticholinesterase activity evaluation of ent-kaurane diterpenoids from *Sideritis arguta*. *J. Nat. Prod.* **2009,** *72 (3),* 500-502.
[22] Culhaoglu, B.; Yapar, G.; Dirmenci, T.; Topcu, G., Bioactive constituents of *Salvia chrysophylla* stapf. *Nat. Prod. Res.* **2013,** *27 (4-5),* 438-447.
[23] Culhaoglu, B.; Hatipoglu, S. D.; Donmez, A. A.; Topcu, G., Antioxidant and anticholinesterase activities of lupane triterpenoids and other constituents of *Salvia trichoclada. Med. Chem. Res.* **2015,** *24 (11),* 3831-3837.
[24] Culhaoglu, B.; Topcu, G., Biologically active lupane triterpenoids from anatolian salvia species. *Planta Med.* **2011,** *77 (12),* 1442-1442.
[25] Culhaoglu, B.; Sener, L. T.; Birman, H.; Albeniz, I.; Topcu, G., Cytotoxic effects of ursolic acid and its derivative on breast cancer cell lines. *Acta Physiol.* **2016,** *218*, 37-37.
[26] Topcu, G.; Kolak, U.; Ozturk, M.; Boga, M.; Hatipoglu, S. D.; Bahadori, F.; Culhaoglu, B.; Dirmenci, T., Investigation of anticholinesterase activity of a series of Salvia extracts and the constituents of *Salvia staminea. Nat. Prod. J.* **2013,** *3*, 3-9.
[27] Ulubelen, A.; Topcu, G., Flavonoids and terpenoids from *Salvia verticillata* and *Salvia pinnata. J. Nat. Prod.* **1984,** *47 (6),* 1068-1068.
[28] Ulubelen, A.; Topcu, G., Triterpenoids from *Salvia pinnata. Phytochemistry* **1984,** *23 (1),* 133-134.
[29] Topcu, G.; Turkmen, Z.; Ulubelen, A.; Schilling, J. K.; Kingston, D. G. I., Highly hydroxylated triterpenes from *Salvia kronenburgii. J. Nat. Prod.* **2004,** *67 (1),* 118-121.
[30] Topcu, G.; Ulubelen, A., Terpenoids from *Salvia kronenburgii. J. Nat. Prod.* **1999,** *62 (12),* 1605-1608.
[31] Erman, B.; Topcu, G. Inhibition of NF-κB activity by triterpene compounds. WO2012130481A1, **2012.**
[32] Hsu, Y. L.; Kuo, P. L.; Lin, C. C., The proliferative inhibition and apoptotic mechanism of saikosaponin d in human non-small cell lung cancer A549 cells. *Life Sci.* **2004,** *75 (10),* 1231-1242.

[33] Reiner, T.; Parrondo, R.; de las Pozas, A.; Palenzuela, D.; Perez-Stable, C., Betulinic acid selectively increases protein degradation and enhances prostate cancer-specific apoptosis: Possible role for inhibition of deubiquitinase activity. *Plos One* **2013,** *8 (2)*,ID: e56234, DOI: 10.1371/journal.pone.0056234, 11 pages.
[34] Salminen, A.; Lehtonen, M.; Suuronen, T.; Kaarniranta, K.; Huuskonen, J., Terpenoids: Natural inhibitors of NF-Kappa B signaling with anti-inflammatory and anticancer potential. *Cell. Mol. Life Sci.* **2008,** *65 (19*), 2979-2999.
[35] Ling, H.; Jia, X. B.; Zhang, Y. C.; Gapter, L. A.; Lim, Y. S.; Agarwal, R.; Ng, K. Y., Pachymic acid inhibits cell growth and modulates arachidonic acid metabolism in nonsmall cell lung cancer a549 cells. *Mol. Carcinog.* **2010,** *49 (3)*, 271-282.
[36] Kaya, B.; Kaplancıklı, Z. A.; Yurttaş, L.; Çiftçi, G. A., Synthesis and biological evaluation of some new pyrimidine bearing 2,5-disubstituted 1,3,4-oxadiazole derivatives as cytotoxic agents. *Turkish J. Biochem.* **2017,** *42 (2)*, 131-137.
[37] Perrin, D. D.; Armarego, W. L., *Purification of laboratory chemicals*. 3th. Ed ed.; 1993.
[38] Senol, H.; Bayrak, C.; Menzek, A.; Sahin, E.; Karakus, M., Cycloaddition reaction of spiro[2.4]hepta-4,6-dien-1-ylmethanol and ptad: A new rearrangement. *Tetrahedron* **2016,** *72 (20)*, 2587-2592.
[39] Bayrak, C.; Senol, H.; Sirtbasi, S.; Sahin, E.; Menzek, A., Synthesis and rearrangement reactions of 1,4-dihydrospiro[1,4-methanonaphthalene-9,1'-cyclopropane] derivatives. *Tetrahedron* **2018,** *74 (40)*, 5839-5849.