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Synthesis and cytotoxic effect of pregnenolone derivatives with one or two α,β-unsaturated carbonyls and an ester moiety at C-21 or C-3

Alejandra Chávez-Riveros[a,*], Abigail Cruz Noriega[b], María Teresa Ramírez Apan[a], Luis D. Miranda[a], Eugene Bratoeff[b,1]

[a] Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510 Cd.Mx., Mexico
[b] Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de Mexico, 04510 Cd.Mx., Mexico

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ABSTRACT

Four series of pregnenolone derivatives having one or two α,β-unsaturated carbonyls and an ester moiety at C-21 or C-3 were synthetized to compare their cytotoxicity effect. The final compounds were evaluated on three human cancer cell lines: PC-3 (prostate cancer), MCF-7 (breast cancer), SKLU-1 (lung cancer) and a non-cancerous cell line HGF (human gingival fibroblast). Two steroids with a 4-fluorinated benzoic acid ester at C-21 were the most active against lung cancer cell line with IC_{50} of 13.1 ± 1.2 and 12.8 ± 0.5 μM and showed a low percentage of cytotoxicity for noncancerous cells (27.63 ± 2.3 and 18.39 ± 1.2% in the screening at 50 μM).

1. Introduction

Cancer is still one of the major public health problem in the world, resulting the leading cause of death. Breast cancer is the most common cancer for women whereas prostate cancer is for men. Lung cancer is the main cause of death for both gender, more than 50% of patients will eventually die of the disease. These three malignancies have the highest incidence and death rate [1,2]. For this reason, the search of new treatment alternatives is crucial.

The structural modification of steroid molecules has demonstrated to be a powerful tool to obtain compounds with a notable improvement in their biological activities, especially for cancer treatment [3–9]. The main characteristics of this kind of structures is their good bioavailability, less toxicity and low vulnerability to multi-drug resistance [10,11].

Pregnenolone, a naturally occurring steroid, is a precursor to several hormones including cortisone, estrogen, testosterone and progesterone [12–14]. Different pregnenolone derivatives have been synthesized and evaluated for various biological activities including as potential anticancer agents [15–17].

Many of the natural or synthetic bioactive steroids have an α,β-unsaturated carbonyl in their structure, such as testosterone, progesterone, finasteride, dexamethasone, cortisol etc. This conjugation, undoubtedly plays an important role in the union with active sites, conferring them remarkable biological activities. We have designed steroidal derivatives containing this scaffold, these compounds reported by our group have displayed cytotoxicity in cancer cell lines and high activity as inhibitors of 5α-reductase enzyme [18–21].

In this work, we present four series of derivatives: Steroids **10a-e** with two carbonyls at C-3 and C-6 conjugated with Δ4 double bond and the ester attached at C-21; and compounds **11c-g** containing just one α,β-unsaturated carbonyl group at C-3 and the ester at C-21. Derivatives **13a-e** and **14a-e** having one α,β-unsaturated carbonyl at C-6 and an aliphatic ester at C-3 (Fig. 1). With this design we wanted to make a comparison between the two extreme positions of the steroid; first comparing the effect of the ester attached at C-21 or C-3 and on the other hand the effect of the enone with one or two carbonyl groups. This study is a preliminary approximation to the structure-activity relationship between the pregnenolone derivatives and their cytotoxicity on cancer cell lines.

2. Experimental

2.1. General

Reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on a Fisher Johns melting point apparatus and were uncorrected. Purity of series **10**, **11**, **13** and **14** was obtained by HPLC on a Waters e2695 equipment using an Eclipse XDB-C18 5 μm 4.6 × 150 mm column at 238 nm. ^{1}H and ^{13}C NMR were taken on an Inova Varian 400 MHz. Chemical shifts are given in ppm relative to that of TMS ($\delta = 0$) in

* Corresponding author.
E-mail address: chavez-riveros@iquimica.unam.mx (A. Chávez-Riveros).
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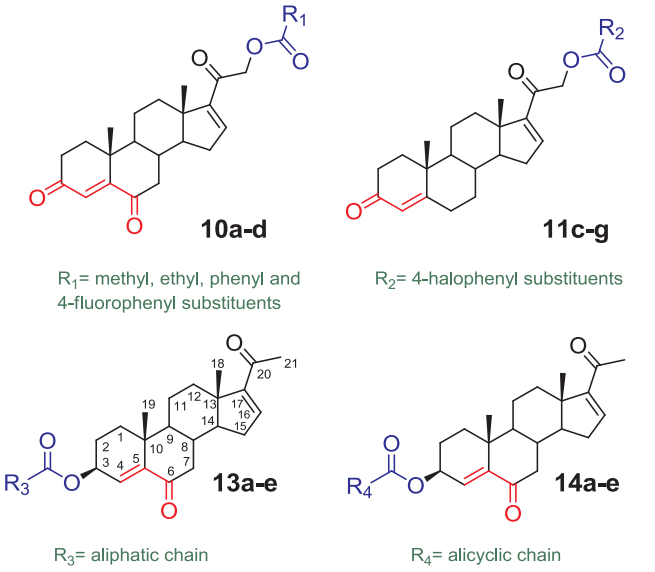


Fig. 1. Position of the conjugated carbonyl group (red) and ester moiety (blue) in the synthesized series. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$CDCl_3$. Low Resolution Mass Spectra (LRMS) were obtained with a Thermo DFS spectrometer by direct infusion and using FAB^+ ionization mode and High Resolution Mass Spectra (HRMS) were recorded with an AccuTOF LC equipment with an ionSense DART controller ionization source. IR spectra were recorded on a PerkinElmer 200 spectrometer.

2.2. Synthesis of series **10a-e** and **11a-e**

2.2.1. 16α,17α-Epoxy-3β-hydroxypregna-5-en-20-one (**2**)

The 16-dehydropregnenolone acetate (1 g, 2.8 mmol) was dissolved in MeOH (66 mL) and a 4 N aqueous solution of NaOH (2 mL) was added. To this mixture, a 30% hydrogen peroxide solution (4 mL) was added drop by drop. The reaction was stirred at room temperature for 4 h. The solvent was evaporated, and cold water was added. The precipitate was filtered and washed with water (2 × 10 mL). The crude was purified by recrystallization from methanol to afford 0.87 g of the pure compound. Yield: 95%, mp 180–182 °C. IR (cm^{-1}): 3454, 2936, 1692, 1642, 1042. ^{1}H NMR ($CDCl_3$) δ: 1.05 (s, 3H, H-18), 1.02 (s, 3H, H-19), 2.2 (m, 2H, H-21), 2.3 (m, 1H, H-16), 3.5 (m, 1H, H-3), 3.68 (s, 1H, –OH), 5.33 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 15.16 (C-18), 19.30 (C-19), 20.01, 26.62, 27.51 (C-21), 29.23, 32.43, 32.51, 37.57, 42.06, 42.16, 45.31, 49.73, 60.49 (C-16), 60.61, 71.02 (C-17), 71.60 (C-3), 120.95 (C-6), 141.12 (C-5), 204.90 (C-20). LRMS FAB^+: 331 $[M+H]^+$.

2.2.2. 16α,17α-Epoxy-3β-tert-butyldimethylsilyloxypregna-5-en-20-one (**3**)

Compound **2** (1 g, 3 mmol) was dissolved in 15 mL of DMF, *tert*-butyldimethylsilyl chloride (0.76 g, 6.8 mmol) and imidazole (0.450 g, 5 mmol) were added to this solution. The mixture was stirred at room temperature for 2 h. After completion of the reaction, the solvent was evaporated and water was added. The precipitate was filtered and washed with water (2 × 20 mL). The crude was purified by column chromatography using a mixture of 20% ethyl acetate in hexane to give 1.3 g of the pure compound **3**. Yield: 98%, mp 126–127 °C. IR (cm^{-1}): 2929, 1698, 1659, 1083. ^{1}H NMR ($CDCl_3$) δ: 0.58 (m, 6H, ($C\underline{H}_3$)$_2$Si), 0.89 (s, 9H, ($C\underline{H}_3$)$_3$CSi), 1.0 (s, 3H, H-18), 1.04 (s, 3H, H-19), 2.3 (s, 3H, H-21), 2.2(m, 1H, H-16), 3.4 (m, 1H, H-3), 5.24 (d, J = 4.9 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: −0.51, 15.18 (C-18), 17.98, 19.38 (C-19), 21.51, 25.81, 25.93 (($C\underline{H}_3$)$_3$CSi), 27.50 (C-21), 30.02, 31.49 (C-Si), 32.04 37.48, 41.90, 43.26, 43.02, 45.92, 51.18, 60.52 (C-16), 71.07 (C-17), 72.49 (C-3), 120.47 (C-6), 141.91 (C-5), 204.89 (C-20). LRMS FAB^+: 445 $[M+H]^+$.

2.2.3. 16α,17α-Epoxy-21-hydroxy-20,20-dimethoxy-3β-tertbutyldimethylsilyloxypregna-5-en-20-one (**4**)

Compound **3** (1 g, 2.25 mmol) was dissolved in 10 mL of dichloromethane; 20 mL of a methanolic solution of NaOH (1 g, 25 mmol) and (diacetoxyiodo)benzene (1.2 g, 3.72 mmol) were added. The mixture was stirred for 18 h. The solvent was evaporated in vacuo, water was added (2 × 20 mL), and the precipitate was filtered and washed with water (2 × 20 mL). The crude was purified on silica gel using 10% ethyl acetate in hexane to give 0.74 g of the pure compound **4**. Yield: 65%, mp 205–207 °C. IR (cm^{-1}): 3596, 1666, 1070, 1033. ^{1}H NMR ($CDCl_3$) δ: 0.05 (m, 6H, ($C\underline{H}_3$)$_2$Si), 0.89 (s, 9H, ($C\underline{H}_3$)$_3$CSi), 1.02 (s, 3H, H-18), 1.26 (s, 3H, H-19), 2.2(m, 1H, H-16), 3.24 (m, 1H, H-3), 3.25 (m, 6H, $C\underline{H}_3$O–), 3.46 (s, 1H, –OH), 3.64 (d, J = 6 Hz, 1H, H-21), 5.31 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: −4.96, 15.10 (C-18), 19.34 (C-19), 25.95 (($C\underline{H}_3$)$_3$CSi), 27.12, 31.55 (C-Si), 32.60, 36.76, 36.98, 42.10, 43.71, 46.26, 49.63 (C-16), 50.34 ($C\underline{H}_3$OH–), 58.33, 62.64 (C-21), 69.65 (C-17), 72.51 (C-3), 101.86 (C-20), 120.70 (C-6), 141.81 (C-5). LRMS FAB^+: 507 $[M+H]^+$.

2.2.4. General methodology for the synthesis of compounds **6a-g**

16α,17α-Epoxy-21-hydroxy-20,20-dimethoxy-3β-*tert*-butyldimethylsilyloxypregna-5-en-20-one **4** (1 eq.) was dissolved in CH_2Cl_2 (0.05 M). DCC (6.7 eq.), DMAP (6.7 eq.) and the corresponding carboxylic acid (6.7 eq.) were added. The resulting solution was stirred at room temperature for 4 h. Ethyl acetate (100 mL) was added and the precipitated dicyclohexyl urea was filtered. The organic phase was washed with 10% aqueous hydrochloric acid (3 × 30 mL), 5% aqueous sodium bicarbonate (3 × 30 mL) and water (3 × 30 mL), the solvent was dried over anhydrous sodium sulfate and evaporated under vacuum to get compound **5**. The crude ester **5** was dissolved in acetone (0.1 M), and to this solution hydrochloric acid (30%) was added drop by drop and the mixture was stirred for 15 min, then water was added and the precipitate was filtered and washed with water. The crude was purified by column chromatography using 10% ethyl acetate in hexane to obtain series **6a-g**.

2.2.4.1. 21-Acetoxy-16α,17α-epoxy-3β-hydroxy-5-pregnen-20-one (**6a**).

Yield: 87%, mp 174–176 °C. IR (cm^{-1}): 3566, 2943, 1754, 1380, 1058. ^{1}H NMR ($CDCl_3$) δ ppm 1.02 (s, 3H, H-18), 1.11 (s, 3H, H-19), 2.16 (s, 3H, –COO$C\underline{H}_3$), 3.52 (m, 1H, H-3), 3.79 (s, 1H, –OH), 4.62 (d, J = 16 Hz, 1H, H-21), 4.70 (d, J = 16 Hz, 1H, H-21), 5.33 (s, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.13 (C-18), 19.32 (C-19), 20.38 ($C\underline{H}_3$COO–), 27.48, 29.61, 31.50, 36.24, 36.32, 41.98, (C-4), 45.56, 45.71, 50.11, 61.55 (C-21), 65.05 (C-16), 70.56 (C-17), 71.58 (C-3), 120.89 (C-6), 141.11 (C-5), 170.41 ($CH_3\underline{C}$OO–), 199.16 (C-20). LRMS FAB^+: 389 $[M+H]^+$.

2.2.4.2. 16α,17α-Epoxy-3β-hydroxy-21-propionoxy-5-pregnen-20-one (**6b**).

Yield: 80%, mp 186–187 °C. IR (cm^{-1}): 3326, 2930, 1744, 1379, 1057. ^{1}H NMR ($CDCl_3$) δ: 1.03 (s, 3H, H-18), 1.12 (s, 3H, H-19), 2.28 (m, 3H, –COOC$H_2C\underline{H}_3$), 2.24 (m, 2H, –COO$C\underline{H}_2$CH$_3$), 3.49 (m, 1H, H-3), 3.8 (s, 1H, –OH), 4.63 (d, J = 20 Hz, 1H, H-21), 4.71 (d, J = 20 Hz, 1H, H-21), 5.31 (s, H-6). ^{13}C NMR ($CDCl_3$) δ: 8.98 (–COOC$H_2\underline{C}$H$_3$), 15.14 (C-18), 19.32 (C-19), 20.15 (–COO$C\underline{H}_2$CH$_3$), 27.10, 29.71, 32.89, 35.70, 37.96, 42.35, 42.41 (C-4), 45.38, 45.61, 49.96, 61.54 (C-21), 65.78 (C-16), 70.57 (C-17), 71.58 (C-3), 120.91 (C-6), 141.10 (C-5), 173.88 (–$\underline{C}$OOCH$_2$CH$_3$), 199.26 (C-20). LRMS FAB^+: 403 $[M+H]^+$.

2.2.4.3. 21-Benzoyloxy-16α,17α-epoxy-3β-hydroxy-5-pregnen-20-one (**6c**).

Yield: 82%, mp 202–203 °C. IR (cm^{-1}): 3484, 3206, 1739, 1379, 1051. ^{1}H NMR ($CDCl_3$) δ: 1.02 (s, 3H, H-18), 1.13 (s, 3H, H-19), 3.52 (m, 1H, H-3), 3.87 (s, 1H, –OH), 4.86 (d, J = 16 Hz, 1H, H-21), 4.94 (d, J = 16 Hz, 1H, H-21), 5.33 (s, 1H, H-6), 7.45 (t, J = 8 Hz, 2H, H-Ar),

7.58 (t, J = 8 Hz, 1H, H-Ar), 8.08 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.15 (C-18), 19.30 (C-19), 20.03, 24.43, 27.86, 28.16, 29.63, 33.82, 37.30, 42.18 (C-4), 45.23, 50.11, 61.58 (C-21), 66.33 (C-16), 70.03 (C-17), 71.58 (C-3), 120.88 (C-6), 126.40 (C-Ar), 128.40 (C-Ar), 129.85 (C-Ar), 133.31 (C-Ar), 141.12 (C-5), 165.99(–COO) 198.94 (C-20). LRMS FAB$^+$: 451 $[M+H]^+$.

*2.2.4.4. 21-(p-Fluoro)benzoyloxy-16α,17α-epoxy-3β-hydroxy-5-pregnen-20-one (**6d**).* Yield: 82%, mp 210–212 °C. IR (cm^{-1}): 3320, 3044, 2936, 1721, 1381, 1055. ^{1}H NMR ($CDCl_3$) δ: 1.02 (s, 3H, H-18), 1.13 (s, 3H, H-19), 3.48 (m, 1H, H-3), 3.87 (s, 1H, –OH), 4.85 (d, J = 16 Hz, 1H, H-21), 4.94 (d, J = 16 Hz, 1H, H-21), 5.33 (s, 1H, H-6), 7.12 (t, J = 12 Hz, 2H, H-Ar), 8.1 (t, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.17 (C-18), 19.33 (C-19), 20.10, 27.89, 29.91, 31.84, 31.96, 37.26, 42.18 (C-4), 42.35, 45.47, 50.09, 61.66 (C-21), 66.44 (C-16), 70.66 (C-17), 71.60 (C-3), 115.74 (C-Ar), 120.91 (C-6), 125.54 (C-Ar), 132.53 (C-Ar), 141.13 (C-5), 165.03 (–COO), 167.26 (C-Ar), 198.85 (C-20). LRMS FAB$^+$: 469 $[M+H]^+$.

*2.2.4.5. 21-(p-Chloro)benzoyloxy-16α,17α-epoxy-3β-hydroxyi-5-pregnen-20-one (**6e**).* Yield: 81%, mp 164–166 °C. IR (cm^{-1}): 3366, 3210, 2927, 1733, 1380, 1067. ^{1}H NMR ($CDCl_3$) δ: 1.02 (s, 3H, H-18), 1.13 (s, 3H, H-19), 3.52 (m, 1H, H-3), 3.87 (s, 1H, –OH), 4.84 (d, J = 20 Hz, 1H, H-21), 4.94 (d, J = 16 Hz, 1H, H-21), 5.35 (s, 1H, H-6), 7.43 (d, J = 8 Hz, 2H, H-Ar), 8.1 (d, J = 12 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.15 (C-18), 19.31 (C-19), 20.08, 27.98, 29.89, 33.75, 34.15, 42.23, 42.50 (C-4), 45.32, 50.46, 61.62 (C-21), 66.48 (C-16), 70.65 (C-17), 71.61 (C-3), 120.90 (C-6), 127.77 (C-Ar), 128.77 (C-Ar), 131.24 (C-Ar), 139.82 (C-Ar), 141.11 (C-5), 165.12 (–COO), 198.78 (C-20). LRMS FAB$^+$: 485 $[M+H]^+$.

*2.2.4.6. 21-(p-Bromo)benzoyloxy-16α,17α-epoxy-3β-hydroxy-5-pregnen-20-one (**6f**).* Yield: 83%, mp 172–174 °C**.** IR (cm^{-1}): 3567, 3322, 2926, 1754, 1380, 1058. ^{1}H NMR ($CDCl_3$) δ: 1.02 (s, 3H, H-18), 1.11 (s, 3H, H-19), 3.52 (m, 1H, H-3), 3.78 (s, 1H, –OH), 4.61 (d, J = 16 Hz, 1H, H-21), 4.69 (d, J = 20 Hz, 1H, H-21), 5.33 (s, 1H, H-6), 7.6 (d, J = 12 Hz, 2H, H-Ar), 7.95 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.14 (C-18), 19.33 (C-19), 20.06, 27.79, 29.86, 31.84, 31.99, 37.12, 42.20 (C-4), 42.47, 45.38, 50.73, 61.55 (C-21), 65.92 (C-16), 70.58 (C-17), 71.65 (C-3), 120.94 (C-6), 128.62 (C-Ar), 131.61 (C-Ar), 131.80 (C-Ar), 141.12 (C-5), 170.37 (–COO), 199.12 (C-20). LRMS FAB$^+$: 529 $[M+H]^+$.

*2.2.4.7. 21-(p-Iodo)benzoyloxy-16α,17α-epoxy-3β-hydroxy-5-pregnen-20-one (**6g**).* Yield: 82%, mp 216–218 °C. IR (cm^{-1}): 3474, 3324, 2931, 1721, 1379, 1056. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.02 (s, 3H, H-18), 1.13 (s, 3H, H-19), 3.53 (m, 1H, H-3), 3.86 (s, 1H, –OH), 4.84 (d, J = 16 Hz, 1H, H-21), 4.94 (d, J = 16 Hz, 1H, H-21), 5.34 (s, 1H, H-6), 7.8 (m, 4H, H-Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 15.17 (C-18), 19.34 (C-19), 20.13, 27.86, 30.02, 31.96, 42.21 (C-4), 42.57, 45.61, 50.03, 58.13, 61.64 (C-21), 66.51 (C-16), 70.68 (C-17), 71.65 (C-3), 101.25 (C-Ar), 120.93 (C-6), 128.83 (C-Ar), 131.26 (C-Ar), 137.81 (C-Ar), 141.13 (C-5), 165.54 (–COO), 198.75 (C-20). LRMS FAB$^+$: 577 $[M+H]^+$.

*2.2.5. General methodology for the synthesis of compounds **7a-g***

Compound **6** (1 eq.) was dissolved in acetone (0.02 M), $CrCl_2$ (5.25 eq.) and acetic acid were added (2%) and the mixture was stirred at room temperature for 45 min. Ice and water (100 mL) were then added and the precipitate was filtered. The crude was purified on silica gel using 10% ethyl acetate in hexane.

*2.2.5.1. 21-Acetoxy-3β-hydroxy-5,16-pregnadien-20-one (**7a**).* Yield: 65%, mp 156–157 °C. IR (cm^{-1}): 3464, 2935, 1729, 1677. ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 3H, H-18), 1.03 (s, 3H, H-19), 2.16 (s, 3H, –COOC$\underline{H}_3$), 3.51 (m, 1H, H-3), 4.87 (d, J = 16 Hz, 1H, H-21), 5.01 (d, J = 16 Hz, 1H, H-21), 5.35 (s, 1H, H-6), 6.74 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 15.83 (C-18), 19.28 (C-19), 20.53 ($\underline{C}H_3COO$–), 29.96, 31.29, 32.26, 34.27, 36.54, 37.09, 42.06, 46.99, 50.96, 56.23, 65.62 (C-21), 71.64 (C-3), 120.90 (C-6), 141.37 (C-5), 144.07 (C-16), 151.96 (C-17), 170.44 ($CH_3\underline{C}OO$–), 190.54 (C-20). LRMS FAB$^+$: 373 $[M+H]^+$.

*2.2.5.2. 3β-Hydroxy-21-propanoyloxy-5,16-pregnadien-20-one (**7b**).* Yield: 60%, mp 183–185 °C. IR (cm^{-1}): 3486, 2929, 1732, 1676. ^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 3H, H-18), 1.03 (s, 3H, H-19), 1.18 (m, 3H, –COOCH$_2$C$\underline{H}_3$), 2.48 (m, 2H, –COOC$\underline{H}_2$CH$_3$), 3.51 (m, 1H, H-3), 4.2 (s, 1H, –OH), 4.88 (d, J = 16 Hz, 1H, H-21), 5.03 (d, J = 16 Hz, 1H, H-21), 5.34 (s, 1H, H-6), 6.75 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 9.03 (COO$\underline{C}$H$_2$CH$_3$), 15.84 (C-18), 19.29 (C-19), 20.88, 27.19 ($\underline{C}$H$_3$CH$_2$COO–), 30.04, 31.96, 32.90, 33.83, 34.09, 42.38, 46.83, 50.46, 56.19, 65.49 (C-21), 71.67 (C-3), 120.91 (C-6), 141.37 (C-5), 143.92 (C-16), 152.02 (C-17), 173.86 (CH$_3$CH$_2\underline{C}$OO–), 190.69 (C-20). LRMS FAB$^+$: 387 $[M+H]^+$.

*2.2.5.3. 21-Benzoyloxy-3β-hydroxy-5,16-pregnadien-20-one (**7c**).* Yield: 61%, mp 163–165 °C. IR (cm^{-1}): 3484, 2932, 1730, 1675. ^{1}H NMR ($CDCl_3$) δ: 0.97 (s, 3H, H-18), 1.04 (s, 3H, H-19), 3.53 (m, 1H, H-3), 4.17 (s, 1H, –OH), 4.62 (d, J = 16 Hz, 1H, H-21), 5.13 (d, J = 16 Hz, 1H, H-21), 5.37 (s, 1H, H-6), 6.84 (s, 1H, H-16), 7.45 (t, J = 12 Hz, 2H, H-Ar), 7.58 (t, J = 8 Hz, 1H, H-Ar), 8.11 (d, J = 12 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.94 (C-18), 19.35 (C-19), 20.08, 30.86, 32.04, 33.58, 34.16, 36.92, 37.78, 42.97, 46.22, 51.43, 56.25, 66.12 (C-21), 71.72 (C-3), 120.99 (C-6), 128.43 (C-Ar), 129.96 (C-Ar), 133.30 (C-Ar), 141.41 (C-5), 144.24 (C-16), 152.08 (C-17), 166.11 (–COO), 190.53 (C-20). LRMS FAB$^+$: 435 $[M+H]^+$.

*2.2.5.4. 21-(p-Fluoro)benzoyloxy-3β-hydroxy-5,16-pregnadien-20-one (**7d**).* Yield: 69%, mp 280–282 °C. IR (cm^{-1}): 3325, 2933, 1723, 1677. ^{1}H NMR ($CDCl_3$) δ: 0.95 (s, 3H, H-18), 1.03 (s, 3H, H-19), 3.51 (m, 1H, H-3), 3.85 (s, 1H, –OH), 5.11 (d, J = 16 Hz, 1H, H-21), 5.25 (d, J = 16 Hz, 1H, H-21), 5.34 (s, 1H, H-6), 6.82 (s, 1H, H-16), 7.11 (t, J = 8 Hz, 2H, H-Ar), 8.11 (t, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.88 (C-18), 19.29 (C-19), 20.49, 30.18, 31.93, 33.52, 34.69, 36.87, 37.75, 42.16, 46.56, 50.06, 56.14, 66.09 (C-21), 71.68 (C-3), 115.67 (C-Ar), 120.93 (C-6), 125.70 (C-Ar), 132.45 (C-Ar), 141.34 (C-5), 144.26 (C-16), 152.00 (C-17), 165.07 (–COO), 167.21 (C-Ar) 190.31 (C-20). LRMS FAB$^+$: 453 $[M+H]^+$.

*2.2.5.5. 21-(p-Chloro)benzoyloxy-3β-hydroxy-5,16-pregnadien-20-one (**7e**).* Yield: 60%, mp 168–170 °C. IR (cm^{-1}): 3252, 2934, 1723, 1670. ^{1}H NMR ($CDCl_3$) δ: 0.96 (s, 3H, H-18), 1.04 (s, 3H, H-19), 3.53 (m, 1H, H-3), 3.86 (s, 1H, –OH), 5.12 (d, J = 16 Hz, 1H, H-21), 5.26 (d, J = 16 Hz, 1H, H-21), 5.37 (s, 1H, H-6), 6.83 (s, 1H, H-16), 7.43 (d, J = 8 Hz, 2H, H-Ar), 8.04 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.88 (C-18), 19.29 (C-19), 20.50, 30.19, 31.95, 33.63, 34.71, 36.92, 37.76, 42.18, 46.58, 50.10, 56.17, 66.15 (C-21), 71.68 (C-3), 120.92 (C-6), 127.93 (C-Ar), 128.74 (C-Ar), 131.29 (C-Ar), 139.73 (C-Ar), 141.35 (C-5), 144.28 (C-16), 152.01 (C-17), 165.19 (–COO), 190.19 (C-20). LRMS FAB$^+$: 469 $[M+H]^+$.

*2.2.5.6. 21-(p-Bromo)benzoyloxy-3β-hydroxy-5,16-pregnadien-20-one (**7f**).* Yield, 63%, mp 157–159 °C. IR (cm^{-1}): 3466, 2930, 1729, 1677. ^{1}H NMR ($CDCl_3$) δ: 0.94 (s, 3H, H-18), 1.05 (s, 3H, H-19), 3.51 (m, 1H, H-3), 3.78 (s, 1H, –OH), 4.88 (d, J = 16 Hz, 1H, H-21), 5.03 (d, J = 16 Hz, 1H, H-21), 5.35 (s, 1H, H-6), 6.75 (s, 1H, H-16), 7.6 (d, J = 8 Hz, 2H, H-Ar), 7.95 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.86 (C-18), 19.31 (C-19), 20.61, 30.21, 32.05, 33.53, 34.85, 36.83, 37.82, 42.23, 46.70, 50.22, 56.33, 65.65 (C-21), 71.69 (C-3), 120.94 (C-6), 127.51 (C-Ar), 129.51 (C-Ar), 131.11 (C-Ar), 132.04 (C-.Ar), 141.39 (C-5), 144.03 (C-16), 152.02 (C-17), 170.43 (–COO), 190.55 (C-20). LRMS FAB$^+$: 513 $[M+H]^+$.

2.2.5.7. 21-(p-Iodo)benzoyloxy-3β-hydroxy-5,16-pregnadien-20-one ***(7g)***. Yield: 57%, mp 195–197 °C. IR (cm^{-1}): 3476, 2932, 1735, 1677. ^1H NMR ($CDCl_3$) δ: 0.96 (s, 3H, H-18), 1.04 (s, 3H, H-19), 3.53 (m, 1H, H-3), 3.85 (s, 1H, –OH), 5.12 (d, J = 16 Hz, 1H, H-21), 5.25 (d, J = 16 Hz, 1H, H-21), 5.77 (s, 1H, H-6), 6.82 (s, 1H, H-16), 7.8 (m, 4H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.17 (C-18), 19.31 (C-19), 30.15, 32.20, 33.76, 34.65, 36.71, 37.74, 42.19, 45.95, 50.21, 56.28, 66.19 (C-21), 71.72 (C-3), 101.15 (C-Ar), 120.95 (C-6), 129.00 (C-Ar), 131.33 (C-Ar), 137.77 (C-Ar), 141.37 (C-5), 144.25 (C-16), 152.06 (C-17), 165.59 (–COO), 190.17 (C-20). LRMS FAB$^+$: 561 $[M+H]^+$.

2.2.6. General methodology for the synthesis of compounds ***8a-d***

Compound **7** (1 eq.) and *m*-chloroperbenzoic acid (3 eq.) were dissolved in dichloromethane (0.07 M), the reaction was stirred at room temperature for 45 min. After this, a saturated aqueous solution of sodium bicarbonate (30 mL) containing sodium bisulfite (0.3 g) was added. The reaction mixture was extracted with dichloromethane (3 × 30 mL); the organic phase was washed with water (3 × 30 mL), dried over sodium sulfate, and the solvent was removed under vacuum. The product was recrystallized from methanol.

2.2.6.1. 21-Acetoxy-5α,6α-epoxy-3β-hydroxy-16-pregnen-20-one ***(8a)***. Yield: 78%, mp 191–195 °C. IR (cm^{-1}): 3446, 2936, 1749, 1677, 1076. ^1H NMR ($CDCl_3$) δ: 0.84 (s, 3H, H-18), 1.06 (s, 3H, H-19), 2.14 (s, 3H, –COOC<u>H</u>$_3$), 2.89 (d, J = 4 Hz, 1H, H-6), 3.45 (s, 1H, –OH), 3.86 (m, 1H, H-3), 4.8 (d, J = 16 Hz, 1H, H-21), 4.99 (d, J = 16 Hz, 1H, H-21), 6.69 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 15.74 (C-18), 15.88 (C-19), 20.30 (<u>C</u>H$_3$COO–), 27.86, 27.95, 31.15, 32.26, 32.45, 33.90, 35.13, 39.97, 42.36, 46.77, 55.82, 58.75 (C-6), 65.57 (C-21), 65.8 (C-5), 68.59 (C-3), 143.7 (C-16), 151.78 (C-17), 170.38 (CH$_3$<u>C</u>OO–), 190.48 (C-20). LRMS FAB$^+$: 389 $[M+H]^+$.

2.2.6.2. 5α,6α-Epoxy-3β-hydroxy-21-propanoyloxy-16-pregnen-20-one ***(8b)***. Yield: 70%, mp 219–221 °C. IR (cm^{-1}): 3493, 2929, 1729, 1679, 1056. ^1H NMR ($CDCl_3$) δ: 0.86 (s, 3H, H-18), 1.08 (s, 3H, H-19), 1.17 (m, 3H, –COOCH$_2$C<u>H</u>$_3$), 2.47 (m, 2H, –COOC<u>H</u>$_2$CH$_3$), 2.91 (d, J = 4 Hz, 1H, H-6), 3.47 (s, 1H, –OH), 3.89 (m, 1H, H-3), 4.84 (d, J = 16 Hz, 1H, H-21), 5.03 (d, J = 16 Hz, 1H, H-21), 6.72 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ ppm 9.02 (COO<u>C</u>H$_2$CH$_3$), 15.77 (C-18), 15.90 (C-19), 20.36, 27.18 (<u>C</u>H$_3$ CH$_2$COO–), 27.90, 27.99, 31.18, 32.33, 32.45, 34.26, 35.25, 39.98, 42.70, 55.99, 58.80 (C-6), 65.47 (C-21), 65.80 (C-5), 68.60 (C-3), 143.63 (C-16), 151.84 (C-17), 173.85 (CH$_3$CH$_2$<u>C</u>OO–), 190.66 (C-20). LRMS FAB$^+$: 403 $[M+H]^+$.

2.2.6.3. 21-Benzoyloxy-5α,6α-epoxy-3β-hydroxy-16-pregnen-20-one ***(8c)***. Yield: 75%, mp 194–197 °C. IR (cm^{-1}): 3423, 2928, 1721, 1624, 1087. ^1H NMR ($CDCl_3$) δ: 0.92 (s, 3H, H-18), 1.12 (s, 3H, H-19), 2.95 (d, J = 4 Hz, 1H, H-6), 3.49 (s, 1H, –OH), 3.92 (m, 1H, H-3), 5.13 (d, J = 16 Hz, 1H, H-21), 5.3 (d, J = 16 Hz, 1H, H-21), 6.84 (s, 1H, H-16), 7.48 (t, J = 12 Hz, 2H, H-Ar), 7.61 (t, J = 12 Hz, 1H, H-Ar), 8.13 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.86 (C-18), 15.94 (C-19), 20.42, 28.24, 28.36, 31.47, 32.12, 32.45, 34.66, 35.51, 39.79, 42.56, 46.87, 55.97, 58.90 (C-6), 65.97 (C-21), 66.10 (C-5), 68.56 (C-3), 128.43 (C-Ar), 129.93 (C-Ar), 133.32 (C-Ar), 144.01 (C-16), 151.82 (C-17), 166.08 (<u>C</u>OO–), 190.48 (C-20). LRMS FAB$^+$: 451 $[M+H]^+$.

2.2.6.4. 21-(p-Fluoro)benzoyloxy-5α,6α-epoxy-3β-hydroxy-16-pregnen-20-one ***(8d)***. Yield: 77%, mp 213–215 °C. IR (cm^{-1}): 3517, 2930, 1718, 1678, 1087. ^1H NMR ($CDCl_3$) δ: 0.89 (s, 3H, H-18), 1.1 (s, 3H, H-19), 2.93 (d, J = 4 Hz, 1H, H-6), 3.84 (s, 1H, –OH), 3.91 (m, 1H, H-3), 5.09 (d, J = 16 Hz, 1H, H-21), 5.27 (d, J = 16 Hz, 1H, H-21), 6.8 (s, 1H, H-16), 7.12 (t, J = 8 Hz, 2H, H-Ar), 8.11 (m, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.14 (C-18), 15.91 (C-19), 20.16, 28.11, 28.25, 31.76, 32.05, 32.36, 34.46, 35.32, 39.68, 42.47, 46.76 58.80 (C-6), 65.87 (C-21), 66.08 (C-5), 68.60 (C-3), 115.47 (C-Ar), 125.71 (C-Ar), 132.56 (C-Ar), 143.97 (C-16), 151.81 (C-17), 165.08 (<u>C</u>OO–), 167.22 (C-Ar), 198.8 (C-20). LRMS FAB$^+$: 469 $[M+H]^+$.

2.2.7. General methodology for the synthesis of compounds ***9a-d***

Compound **8** (1 eq.) was dissolved in acetone (0.1 M). An aqueous solution (1.8 M) of chromium trioxide was added dropwise at 0 °C for 10 min. The resulting mixture was allowed to warm up to room temperature and after 30 min the same amount of chromium trioxide was added in the same manner. The mixture was diluted with cold water (75 mL) and the precipitate was filtered and dried. The product was recrystallized from methanol.

2.2.7.1. 21-Acetoxy-5α-hydroxy-16-pregnen-3,6,20-trione ***(9a)***. Yield: 82%, mp 218–220 °C. IR (cm^{-1}): 3346, 2943, 1711, 1681. ^1H NMR ($CDCl_3$) δ: 0.89 (s, 3H, H-18), 1.0 (s, 3H, H-19), 2.14 (s, 3H, –COOC<u>H</u>$_3$), 2.84 (d, J = 16 Hz, 1H, H-4), 2.87 (d, J = 16 Hz, 1H, H-4), 3.78 (s, 1H, –OH), 4.85 (d, J = 16 Hz, 1H, H-21), 4.99 (d, J = 16 Hz, 1H, H-21), 6.71 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 13.75 (C-18), 15.88 (C-19), 20.48 (<u>C</u>H$_3$COO–), 22.31, 31.97, 32.18, 34.16, 35.77, 37.86, 42.09, 43.67, 45.21, 45.79, 55.89, 65.58 (C-21), 82.69 (C-5), 143.44 (C-16), 151.57 (C-17), 170.44 (CH$_3$<u>C</u>OO–), 190.44 (C-20), 210.2 (C-3), 211.13 (C-6). LRMS FAB$^+$: 403 $[M+H]^+$.

2.2.7.2. 5α-Hydroxy-21-propanoyloxy-16-pregnen-3,6,20-trione ***(9b)***. Yield: 76%, mp 288–190 °C. IR (cm^{-1}): 3327, 2943, 1750, 1678. ^1H NMR ($CDCl_3$) δ: 0.86 (s, 3H, H-18), 0.97 (s, 3H, H-19), 1.12 (m, 3H, –COOCH$_2$C<u>H</u>$_3$), 2.4 (m, 2H, –COOC<u>H</u>$_2$CH$_3$), 2.8 (d, J = 16 Hz, 1H, H-4), 2.87 (d, J = 16 Hz, 1H, H-4), 3.75 (s, 1H, –OH), 4.86 (d, J = 16 Hz, 1H, H-21), 4.98 (d, J = 16 Hz, 1H, H-21), 6.67 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 9.95 (COO<u>C</u>H$_2$CH$_3$), 12.80 (C-18), 14.93 (C-19), 23.75, 23.80, 26.19 (<u>C</u>H$_3$ CH$_2$COO–), 31.24, 32.24, 34.22, 35.89, 37.93, 42.26, 43.74, 46.11, 55.90, 64.47 (C-21), 81.79 (C-5), 142.30 (C-16), 150.67 (C-17), 172.80 (CH$_3$CH$_2$<u>C</u>OO–), 189.59 (C-20), 209.05 (C-3), 209.94 (C-6). LRMS FAB$^+$: 417 $[M+H]^+$.

2.2.7.3. 21-Benzoyloxy-5α-hydroxy-16-pregnen-3,6,20-trione ***(9c)***. Yield: 78%, mp 203–205 °C. IR (cm^{-1}): 3324, 2928, 1711, 1679. ^1H NMR ($CDCl_3$) δ: 0.92 (s, 3H, H-18), 1.25 (s, 3H, H-19), 2.88 (s, 2H, H-4), 3.6 (s, 1H, –OH), 5.13 (d, J = 16 Hz, 1H, H-21), 5.27 (d, J = 16 Hz, 1H, H-21), 6.83 (s, 1H, H-16), 7.46 (t, J = 8 Hz, 2H, H-Ar), 7.5 (m, 1H, H-Ar), 8.1 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 14.05 (C-18), 15.95 (C-19), 23.89, 29.22, 29.78, 33.80, 35.66, 36.79, 38.45, 41.88, 44.36, 47.69. 68.15 (C-21), 82.72 (C-5), 128.41 (C-Ar), 128.78 (C-Ar), 129.91 (C-Ar), 130.88 (C-Ar), 132.41 (C-Ar), 133.31 (C-Ar), 143.54 (C-16), 151.66 (C-17), 167.7 (–<u>C</u>OO), 190.38 (C-20), 210.22 (C-3), 210.9 (C-6). LRMS FAB$^+$: 465 $[M+H]^+$.

2.2.7.4. 21-(p-Fluoro)benzoyloxy-5α-hydroxy-16-pregnen-3,6,20-trione ***(9d)***. Yield: 86%, mp 135–136 °C. IR (cm^{-1}): 3332, 2950, 1710, 1685. ^1H NMR ($CDCl_3$) δ: 0.95 (s, 3H, H-18), 1.04 (s, 3H, H-19), 2.8 (m, 2H, H-4), 3.89 (s, 1H, –OH), 5.12 (d, J = 16 Hz, 1H, H-21), 5.26 (d, J = 16 Hz, 1H, H-21), 6.81 (s, 1H, H-16), 7.13 (t, J = 8 Hz, 2H, H-Ar), 8.12 (m, 2H, H-Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 13.82 (C-18), 15.98 (C-19), 20.40, 27.99, 31.78, 32.56, 34.47, 35.79, 41.86, 42.78, 44.46, 47.80, 55.74, 66.10 (C-21), 82.77 (C-5), 115.52 (C-Ar), 125.68 (C-Ar), 132.59 (C-Ar), 143.62 (C-16), 151.68 (C-17), 165.09 (–<u>C</u>OO), 167.28 (C-Ar), 190.26 (C-20), 210.18 (C-3), 210.87 (C-6). LRMS FAB$^+$: 483 $[M+H]^+$.

2.2.8. General methodology for the synthesis of compounds ***10a-d***

Compound **9** (1 eq.) was dissolved in dichloromethane (0.25 M), 0.004 eq. of pyridine was added and the solution was cooled to 0 °C. Thionyl chloride (0.0015 eq.) was added dropwise under nitrogen atmosphere and the resulting solution was stirred at room temperature for 2 h. Iced water (100 mL) was added and the solution was extracted three times with ethyl acetate (25 mL). The organic layer was washed

with 10% aqueous hydrochloric acid (25 mL), 5% aqueous sodium bicarbonate (25 mL), and water (25 mL). The organic layer was dried over sodium sulfate and the solvent was evaporated under vacuum. The product was purified by column chromatography using a mixture of 20% ethyl acetate in hexane.

*2.2.8.1. 21-Acetoxypregna-4,16-dien-3,6,20-trione (**10a**).* Yield: 87%, purity 98%, mp 215–217 °C. IR (cm^{-1}): 2942, 1745, 1677. ^{1}H NMR ($CDCl_3$) δ: 0.98 (s, 3H, H-18), 1.21 (s, 3H, H-19), 2.19 (s, 3H, –COOC$\underline{H}_3$), 4.9 (d, J = 16 Hz, 1H, H-21), 5.3 (d, J = 16 Hz, 1H, H-21), 6.2 (s, 1H, H-4), 6.76 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 15.84 (C-18), 17.45 (C-19), 20.60 ($\underline{C}H_3$COO–), 31.96, 33.83, 35.71, 39.89, 36.26, 36.55, 51.49, 56.17, 65.57 (C-21), 125.81 (C-4), 143.16 (C-16), 151.37 (C-17), 160.41 (C-5), 170.34 ($CH_3\underline{C}$OO–), 190.30 (C-20), 199.18 (C-3), 201.21 (C-6). HRMS $[M+H]^+$ cal for $C_{23}H_{29}O_5$ 385.2015 found 385.2023.

*2.2.8.2. 21-Propanoyloxypregna-4,16-dien-3,6,20-trione (**10b**).* Yield: 83%, purity 99%, mp 212–215 °C. IR (cm^{-1}): 2940, 1743, 1671. ^{1}H NMR ($CDCl_3$) δ: 0.97 (s, 3H, H-18), 1.0 (s, 3H, H-19), 1.12 (m, 3H, –COOCH$_2$C$\underline{H}_3$), 2.4 (m, 2H, –COOC$\underline{H}_2$CH$_3$), 4.8 (d, J = 16 Hz, 1H, H-21), 5.2 (d, J = 16 Hz, 1H, H-21), 6.2 (s, 1H, H-4), 6.67 (s, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 9.95 (COO$\underline{C}$H$_2$CH$_3$), 14.90 (C-18), 16.11 (C-19), 26.26 ($\underline{C}$H$_3$ CH$_2$COO–), 31.88, 33.71, 35.46, 39.86, 36.22, 36.30, 51.27, 56.03, 64.34 (C-21), 125.56 (C-4), 142.12 (C-16), 150.67 (C-17), 160.20 (C-5), 172.85 (CH$_3$CH$_2\underline{C}$OO–), 189.72 (C-20), 199.11 (C-3), 201.06 (C-6). HRMS $[M+H]^+$ cal for $C_{24}H_{31}O_5$ 399.2172 found 399.2181.

*2.2.8.3. 21-Benzoyloxypregna-4,16-dien-3,6,20-trione (**10c**).* Yield: 83%, purity 99%, mp 165–167 °C. IR (cm^{-1}): 2930, 1721, 1683. ^{1}H NMR ($CDCl_3$) δ: 1.13 (s, 3H, H-18), 1.18 (s, 3H, H-19), 5.08 (d, J = 16 Hz, 1H, H-21), 5.2 (d, J = 16 Hz, 1H, H-21), 6.13 (s, 1H, H-4), 6.78 (s, 1H, H-16), 7.36 (s, 2H, H-Ar), 7.52 (m, 1H, H-Ar), 8.05 (s, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 14.89 (C-18), 16.45 (C-19), 19.99, 29.77, 31.47, 32.39, 33.19, 34.22, 37.68, 38.52, 50.08, 54.48, 67.14 (C-21), 124.80 (C-4), 127.43 (C-Ar), 128.89 (C-Ar), 129.81 (C-Ar), 132.36 (C-Ar), 142.37 (C-16), 150.39 (C-17), 159.51 (C-5), 165.01 ($\underline{C}$OO–), 189.33 (C-20), 198.34 (C-3), 200.41 (C-6). HRMS $[M+H]^+$ cal for $C_{28}H_{31}O_5$ 447.2171 found 447.2183.

*2.2.8.4. 21-(p-Fluoro)benzoyloxypregna-4,16-dien-3,6,20-trione (**10d**).* The characterization of this compound was already reported in our previous work [20].

*2.2.9. General methodology for the synthesis of compounds **11c-g***

Compound **7** (1 eq.) was dissolved in toluene (0.025 M), *N*-methyl-4-piperidone (20 eq.) were added. After the addition, 25% of toluene were distilled with a Dean-Stark system to eliminate the water of the reaction. Aluminium isopropoxide (5 eq.) was added and other 25% of toluene were distilled. The reaction was stirred under reflux for 18 h. The mixture was dissolved with 50 mL of ethyl acetate and 30 mL of a 15% aqueous solution of HCl were added, the reaction was stirred for 30 min and the organic layer was separated and washed with a saturated solution of sodium bicarbonate (3 × 30 mL) and water (3 × 30 mL). The organic layer was evaporated and the crude was purified on silica gel using 10% ethyl acetate in hexane getting yields from 58 to 65%.

*2.2.9.1. 21-Benzoyloxypregna-4,16-dien-3,20-dione (**11c**).* Yield: 63%, purity 99%, mp 192–194 °C. IR (cm^{-1}): 2953, 1727, 1672. ^{1}H NMR ($CDCl_3$) δ: 0.98 (s, 3H, H-18), 1.21 (s, 3H, H-19), 5.11 (d, J = 16 Hz, 1H, H-21), 5.27 (d, J = 16 Hz, 1H, H-21), 5.73 (s, 1H, H-4), 6.83 (s, 1H, H-16), 7.4 (t, J = 8 Hz, 2H, H-Ar), 7.57 (t, J = 8 Hz, 1H, H-Ar), 8.09 (t, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.96 (C-18), 20.71 (C-19), 27.38, 31.82, 32.40, 33.19, 33.98, 24.10, 35.66, 38.99, 48.72, 53.95, 55.70, 66.03 (C-21), 124.03 (C-4), 128.38 (C-Ar), 129.89 (C-Ar), 133.26 (C-Ar), 143.74 (C-16), 151.35 (C-17), 166.01 ($\underline{C}$OO–), 170.65 (C-5), 190.38 (C-20), 199.39 (C-3). HRMS $[M+H]^+$ cal for $C_{28}H_{33}O_4$ 433.2372 found 433.2372.

*2.2.9.2. 21-(p-Fluoro)benzoyloxypregna-4,16-dien-3,20-dione (**11d**).* Yield: 61%, purity 98%, mp 148–150 °C. IR (cm^{-1}): 2929, 1728, 1678. ^{1}H NMR ($CDCl_3$) δ: 0.87 (s, 3H, H-18), 1.15 (s, 3H, H-19), 5.15 (d, J = 16 Hz, 1H, H-21), 5.3 (d, J = 16 Hz, 1H, H-21), 5.67 (s, 1H, H-4), 6.63 (s, 1H, H-16), 7.3 (m, 2H, H-Ar), 8.1 (m, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ ppm 14.78 (C-18), 19.73 (C-19), 26.16, 28.85, 28.97, 31.96, 32.31, 32.48, 34.44, 38.05, 48.12, 53.19, 54.93, 67.15 (C-21), 114.45 (C-Ar), 122.97 (C-4), 127.78 (C-Ar), 129.86 (C-Ar), 131.45 (C-Ar), 143.08 (C-16), 154.14 (C-17), 166.74 ($\underline{C}$OO–), 169.91 (C-Ar), 170.10 (C-5), 195.69 (C-20), 198.51 (C-3). HRMS $[M+H]^+$ cal for $C_{28}H_{32}FO_4$ 451.2285 found 451.2298.

*2.2.9.3. 21-(p-Chloro)benzoyloxypregna-4,16-dien-3,20-dione (**11e**).* Yield: 65%, purity 98%, mp 157–159 °C. IR (cm^{-1}): 2931, 1727, 1676. ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, 3H, H-18), 1.15 (s, 3H, H-19), 5.04 (d, J = 16 Hz, 1H, H-21), 5.2 (d, J = 16 Hz, 1H, H-21), 5.67 (s, 1H, H-4), 6.76 (s, 1H, H-16), 7.36 (d, J = 8 Hz, 2H, H-Ar), 7.98 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 14.99 (C-18), 19.73 (C-19), 26.12, 28.46, 28.55, 31.50, 32.05, 32.21, 34.19, 38.00, 48.10, 53.06, 67.16 (C-21), 123.04 (C-4), 127.77 (C-Ar), 129.85 (C-Ar), 130.29 (C-Ar), 142.91 (C-Ar), 143.22 (C-16), 150.80 (C-17), 169.60 ($\underline{C}$OO–), 170.60 (C-5), 196.30 (C-20), 199.10 (C-3). HRMS $[M+H]^+$ cal for $C_{28}H_{32}ClO_4$ 467.1989 found 467.1995.

*2.2.9.4. 21-(p-Bromo)benzoyloxypregna-4,16-dien-3,20-dione (**11f**).* Yield: 60%, purity 99%, mp 161–163 °C. IR (cm^{-1}): 2929, 1727, 1677. ^{1}H NMR ($CDCl_3$) δ ppm 0.94 (s, 3H, H-18), 1.16 (s, 3H, H-19), 5.1 (d, J = 16 Hz, 1H, H-21), 5.25 (d, J = 16 Hz, 1H, H-21), 5.67 (s, 1H, H-4), 6.77 (s, 1H, H-16), 7.6 (d, J = 8 Hz, 2H, H-Ar), 7.7 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 15.10 (C-18), 19.91 (C-19), 26.79, 28.66, 28.79, 31.99, 32.27, 34.61, 38.56, 48.19, 48.30, 53.29, 67.5 (C-21), 124.15 (C-4), 127.18 (C-Ar), 129.09 (C-Ar), 130.17 (C-Ar), 132.44 (C-Ar), 144.32 (C-16), 153.95 (C-17), 166.38 ($\underline{C}$OO–), 170.39 (C-5), 196.40 (C-20), 199.12 (C-3). HRMS $[M+H]^+$ cal for $C_{28}H_{32}BrO_4$ 511.1484 found 511.1495.

*2.2.9.5. 21-(p-Iodo)benzoyloxypregna-4,16-dien-3,20-dione (**11g**).* Yield: 58%, purity 99%, mp 186–188 °C. IR (cm^{-1}): 2932, 1729, 1675. ^{1}H NMR ($CDCl_3$) δ: 0.97 (s, 3H, H-18), 1.15 (s, 3H, H-19), 5.03 (d, J = 16 Hz, 1H, H-21), 5.19 (d, J = 16 Hz, 1H, H-21), 5.66 (s, 1H, H-4), 6.74 (s, 1H, H-16), 7.8 (d, J = 8 Hz, 2H, H-Ar), 7.88 (d, J = 8 Hz, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 14.90 (C-18), 19.60 (C-19), 26.15, 28.33, 28.58, 31.03, 32.45, 34.89, 38.63, 48.59, 48.96, 53.54, 67.00 (C-21), 98.50 (C-Ar), 124.10 (C-4), 129.34 (C-Ar), 130.96 (C-Ar), 137.55 (C-Ar), 144.07 (C-16), 154.11 (C-17), 167.32 ($\underline{C}$OO–), 169.96 (C-5), 197.27 (C-20), 199.09 (C-3). HRMS $[M+H]^+$ cal for $C_{28}H_{32}IO_4$ 559.1345 found 559.1353.

*2.3. General synthesis of compounds **13a-e** and **14a-e***

The synthesis and spectroscopy data of compounds **12**, **13a**, **13b** and **14a-d** were published in our previous work [19,22–24], and just the purity and HRMS is reported here. The Steglich reaction was done following the general procedure: the steroid **12** (1 eq.), DCC (3 eq.), DMAP (3 eq.) and the corresponding carboxylic acid (3 eq.) were dissolved in CH_2Cl_2 (0.09 M) and stirred for 4 h. Chilled ethyl acetate (100 mL) was added and the precipitated dicyclohexyl urea was filtered. The organic layer was washed three times with 10% aqueous hydrochloric acid (50 mL), 5% aqueous sodium bicarbonate (3 × 50 mL) and water (3 × 50 mL). The solution was dried over anhydrous sodium sulfate and the crude was purified by recrystallization from methanol.

*2.3.1. 3β-Acetoxypregna-4,16-dien-6,20-dione **(13a)***

Yield 81%, purity 98%, HRMS $[M+H]^+$ cal for $C_{23}H_{31}O_4$ 371.2222 found 371.2227.

*2.3.2. 3β-Propanoyloxypregna-4,16-dien-6,20-dione **(13b)***

Yield 75%, purity 98%, HRMS $[M+H]^+$ cal for $C_{24}H_{33}O_4$ 385.2379 found 385.2374.

*2.3.3. 3β-Butanoyloxypregna-4,16-dien-6,20-dione **(13c)***

Yield: 73%, purity 99%, mp 142–144 °C. IR (cm^{-1}): 1732, 1688, 1656. ^1H NMR ($CDCl_3$) δ: 0.9 (m, 3H, C$\underline{H}_3$ ester), 0.92 (s, 3H, H-18), 1.3(s, 3H, H-19), 2.2(s, 3H, H-21), 5.3(m, 1H, H-3), 6.0 (s, 1H, H-4), 6.7(m, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 13.62 (C$\underline{H}_3$ ester), 15.75 (C-18), 18.92, 19.59 (C-19), 20.11, 23.70, 27.08, 31.77, 32.03, 33.48, 36.24, 38.22, 38.41, 45.98, 46.13, 51.50, 56.07 (C-21), 68.93 (C-3), 129.27 (C-4), 143.74 (C-16), 147.78 (C-5), 154.89 (C-17), 173.26 (C═O del ester), 196.53 (C-20), 201.78 (C-6). HRMS $[M+H]^+$ cal for $C_{25}H_{35}O_4$ 399.2535 found 399.2519.

*2.3.4. 3β-Pentanoyloxypregna-4,16-dien-6,20-dione **(13d)***

Yield: 69%, purity 99%, mp 125–127 °C. IR (cm^{-1}): 1735, 1688, 1656. ^1H NMR ($CDCl_3$) δ: 0.9 (m, 3H, C$\underline{H}_3$ ester), 0.92 (s, 3H, H-18), 1.3(s, 3H, H-19), 2.2(s, 3H, H-21), 5.3(m, 1H, H-3), 6.0 (s, 1H, H-4), 6.7(m, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 13.71 (C$\underline{H}_3$ ester), 14.11, 15.80 (C-18), 19.64 (C-19), 20.16, 22.15, 24.33, 27.02 (C-21), 31.96, 32.36, 38.48, 41.96, 45.88, 48.77, 48.98, 51.87, 46.20, 60.44, 68.98 (C-3), 129.33 (C-4), 143.77 (C-16), 147.81 (C-5), 154.93 (C-17), 173.50 (C═O ester), 196.59 (C-20), 201.78 (C-6). HRMS $[M+H]^+$ cal for $C_{26}H_{37}O_4$ 413.2691 found 413.2697.

*2.3.5. 3β-Hexanoyloxypregna-4,16-dien-6,20-dione **(13e)***

Yield: 77%, purity 98%, mp 105–106 °C. IR (cm^{-1}): 1726, 1688, 1664. ^1H NMR ($CDCl_3$) δ: 0.9 (m, 3H, C$\underline{H}_3$ del ester), 0.95 (s, 3H, H-18), 1.1(s, 3H, H-19), 2.2(s, 3H, H-21), 5.3(m, 1H, H-3), 6.0 (s, 1H, H-4), 6.7(m, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 13.87 (C$\underline{H}_3$ ester), 15.76 (C-18), 19.59 (C-19), 20.23, 22.05, 22.19, 24.11, 24.16, 27.08 (C-21), 31.77, 31.89, 32.06, 34.40, 38.06, 38.21, 46.20, 50.60, 50.73, 56.27, 68.94 (C-3), 129.29 (C-4), 143.74 (C-16), 147.77 (C-5), 154.89 (C-17), 173.46 (C═O ester), 196.54 (C-20), 201.73 (C-6). HRMS $[M+H]^+$ cal for $C_{27}H_{39}O_4$ 427.2848 found 427.2853.

*2.3.6. 3β-Cyclopropanecarbonyloxypregna-4,16-dien-6,20-dione **(14a)***

Yield 65%, purity 98%, HRMS $[M+H]^+$ cal for $C_{25}H_{33}O_4$ 397.2378 found 397.2386.

*2.3.7. 3β-Cyclobutanecarbonyloxypregna-4,16-dien-6,20-dione **(14b)***

Yield 67%, purity 99%. HRMS $[M+H]^+$ cal for $C_{26}H_{35}O_4$ 411.2535 found 411.2531.

*2.3.8. 3β-Cyclopentanecarbonyloxypregna-4,16-dien-6,20-dione **(14c)***

Yield 70%, purity 99%. HRMS $[M+H]^+$ cal for $C_{27}H_{37}O_4$ 425.2691 found 425.2691.

*2.3.9. 3β-Cyclohexanecarbonyloxypregna-4,16-dien-6,20-dione **(14d)***

Yield 71%, purity 99%. HRMS $[M+H]^+$ cal for $C_{28}H_{39}O_4$ 439.2848 found 4439.2845.

*2.3.10. 3β-Cycloheptanecarbonyloxypregna-4,16-dien-6,20-dione **(14e)***

Yield: 61%, purity 99%, mp 134–137 °C. IR (cm^{-1}): 1727, 1693, 1652. ^1H NMR ($CDCl_3$) δ: 0.93 (s, 3H, H-18), 1.02(s, 3H, H-19), 2.19 (s, 3H, H-21), 2.27 (m, 1H, C$\underline{H}$ ester), 5.33(m, 1H, H-3), 6.06 (s, 1H, H-4), 6.70(m, 1H, H-16). ^{13}C NMR ($CDCl_3$) δ: 14.54 (C-18), 19.65 (C-19), 20.19, 23.86, 27.56, 28.98 (C-21), 31.79, 33.95, 34.04, 38.14, 38.39, 43.24, 43.46, 44.60, 46.39 (C$\underline{H}$ ester), 51.27, 51.53, 53.85, 56.48, 57.83, 68.65 (C-3), 129.55 (C-4), 143.74 (C-16), 147.32 (C-5), 154.89 (C-17), 175.67 (C═O del éster), 196.53 (C-20), 201.44 (C-6). HRMS $[M+H]^+$ cal for $C_{29}H_{41}O_4$ 453.3004 found 4453.3013.

3. Results and discussion

3.1. Chemistry

The commercially available 16-dehydropregnenolone acetate **1** was the starting material for two synthetic routes. To prepare the first series with an α,β-unsaturated dicarbonyl group, compound **2** was synthesized from the starting material **1** using hydrogen peroxide under basic condition to form the 16α,17α-epoxy steroid **3**. The protection of the alcohol **3** with *tert*-butyldimethylsilyl chloride followed by oxidation of C-21 using (diacetoxyiodo)benzene afforded **4** [25]. The alcohol **4** was esterified using the corresponding aliphatic or aromatic carboxylic acid to get **5a-g**. The deprotection of the alcohol and the carbonyl groups of molecules **5a-g** were performed under acid conditions to obtain derivatives **6a-g**. In order to regenerate the double bond at C-16, the epoxide was reduced using $CrCl_2$ [26] to give compounds **7a-g**. Alternatively, compound **7a** could be synthesized from a 21-iodo derivative [27]. For the series **10a-e** the double bond at C-5 of steroids **7a-e** were reacted with *m*-chloroperoxybenzoic acid to afford the 5α,6α-epoxycompounds **8a-e**. Oxidation of the epoxide **8a-e** with chromic acid yielded the 3,6-diketo-5α-dialcohol **9a-e**. Dehydration of the hydroxyl group at C-4 in **9a-e** with thionyl chloride afforded the α,β-unsaturated dicarbonyl analogs **10a-e** with global yields from 10 to 16% (Scheme 1). The series **11c-g** were prepared using the Oppenauer reaction from the alcohol derivatives **7c-g** (Scheme 1). The desired molecules were obtained with global yields of 14–18%.

We reported the methodology and spectroscopy data to obtain derivative **12** in our previous work [19]. The esters at C-3 in compounds **13a-e** and **14a-e** were synthesized from alcohol **12** using the Steglich reaction [28] to afford two groups of derivatives, one with a different size of lineal chain substituents obtained with global yields of 31–50% and the other with alicyclic substituents with global yields ranging from 29 to 32% (Scheme 2).

3.2. Cytotoxic effect

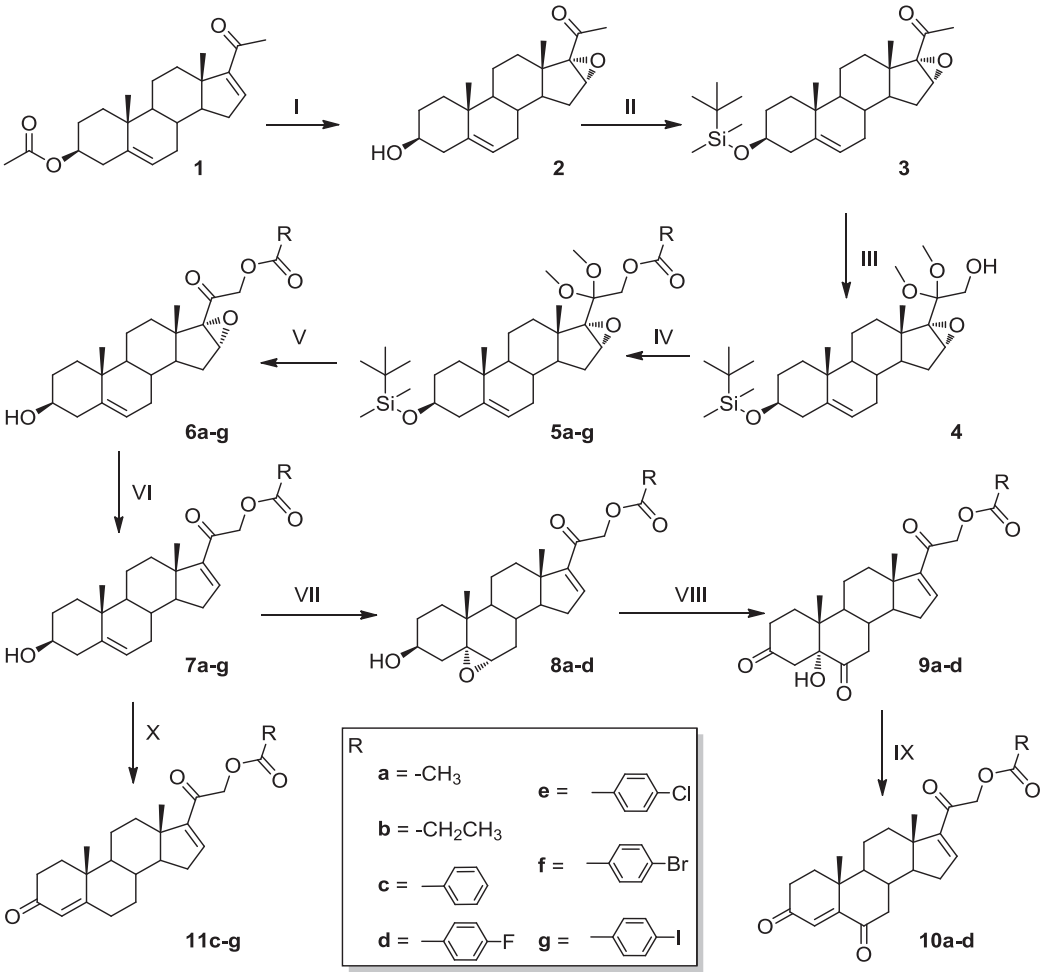
We evaluated the cytotoxic effect of series **10a-d**, **11c-g, 13a-e** and **14a-e**, in a panel of three human cancer cell lines (PC-3, prostatic adenocarcinoma; MCF-7, mammary adenocarcinoma and SKLU-1, lung adenocarcinoma) and one noncancerous cell line (HGF, human gingival fibroblast). The values of the primary screening are showed in Table 1. Etoposide, a glycoside of podophyllotoxin was used as a reference drug.

For molecules with an ester moiety at C-21 (**10a-d** and **11c-g**), derivatives **10d** and **11d** with an aromatic ester having a fluor at *para* position, showed high cytotoxic activity on the three cancer cell lines and a low cytotoxicity on the noncancerous cell line, **11d** had even a lower inhibition percentage than that of the etoposide on HGF.

Steroids **13a-e** with an aliphatic ester at C-3 showed a higher inhibition percentage in the three cancer cell lines when the side chain has even number of carbons (**13a**, **13c**, **13e**) as compare to those with odd number (**13b** and **13d**). Besides, when the aliphatic ester is attached at C-21 (**10a** and **10b**) the same phenomenon is observed, this behavior might be due to the specific orientation of the aliphatic chain at the active site. In the case of compounds **14a-e**, just **14b** (with a cyclobutyl ester) showed good cytotoxicity, especially on SKLU-1 cell line.

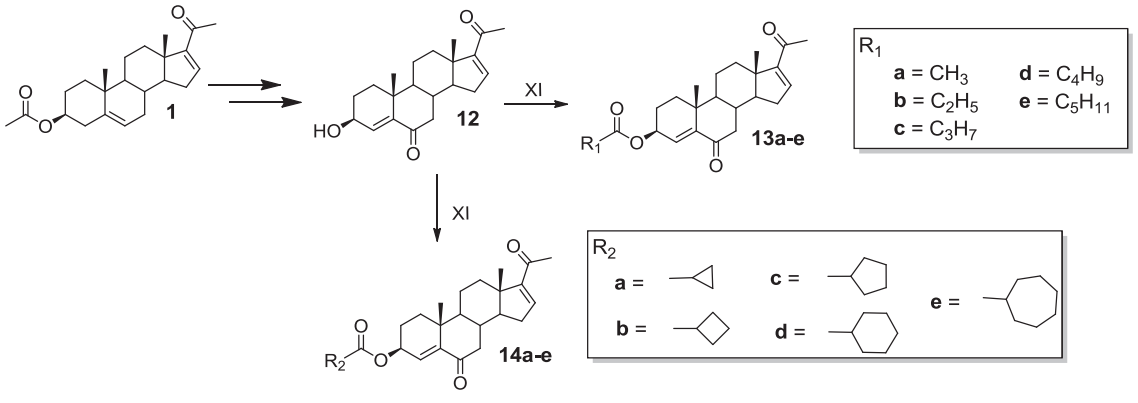
Compounds **10a**, **10d**, **11d**, **13e** and **14b**, were chosen to determine their IC_{50} on the lung cancer cell line and derivatives **10d** and **11d** have the lower IC_{50} (Table 2). This results might be due to fluorine electrostatic interactions [29] but other mechanisms could be implicated.

From this study, we can infer that the steroids with an ester moiety at C-21 present more cytotoxicity on the lung cancer cell line than that



Conditions: I: H_2O_2, NaOH 4N, MeOH **II:** TBDMS, imidazole, DMF **III:** $C_6H_5I(OAc)_2$, NaOH, MeOH **IV:** RCOOH, DCC, DMAP, CH_2Cl_2 **V:** HCl, acetone **VI:** $CrCl_2$, acetic acid, acetone **VII:** *m*-CPBA, CH_2Cl_2 **VIII:** CrO_3, H_2O, acetone **IX:** $SOCl_2$, Py, CH_2Cl_2 **X:** $Al(i\text{-}PrO)_3$, *N*-methyl-4-piperidone, toluene.

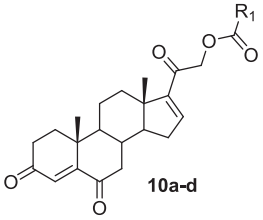
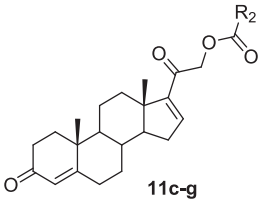
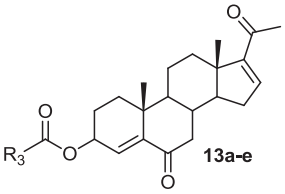
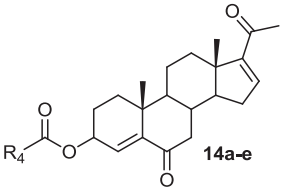
Scheme 1. Synthesis of compounds **10a-d** and **11c-g**.



Conditions: XI: R_1COOH or R_2COOH, DCC, DMAP, CH_2Cl_2.

Scheme 2. Synthesis of compounds **13a-e** and **14a-e**.

Table 1
Preliminary cytotoxicity screening of series **10a–d**, **11c–g**, **13a–e** and **14a–e**, at 50 μM.

Structure	Compound (50 μM)	PC-3	MCF-7	SKLU-1	HGF
Etoposide		44.2 ± 1.2[a]	73.3 ± 1.0[a]	83.5 ± 1.1[a]	24.21 ± 1.1
 10a-d	**10a** (R_1 = CH_3)	49.8 ± 2.9	75.3 ± 2.5	94.2 ± 0.6	12.2 ± 3.8
	10b (R_1 = C_2H_5)	21.5 ± 0.9	56.6 ± 2.8	63.3 ± 4.8	25.6 ± 1.8
	10c (R_1 = C_6H_5)	57.5 ± 3.4	40.2 ± 3.7	67.7 ± 4.8	21.62 ± 2.4
	10d (R_1 = 4-F-C_6H_5)	98.1 ± 1.9	92.2 ± 2.5	100	27.63 ± 2.3
 11c-g	**11c** (R_2 = C_6H_5)	19.0 ± 1.8	25.9 ± 3.6	27.6 ± 3.6	NC[b]
	11d (R_2 = 4-F-C_6H_5)	92.0 ± 4.3	89.1 ± 4.9	98.1 ± 1.9	18.39 ± 1.2
	11e (R_2 = 4-Cl-C_6H_5)	55.9 ± 3.0	43.9 ± 5.8	63.7 ± 1.9	12.8 ± 1.2
	11f (R_2 = 4-Br-C_6H_5)	50.0 ± 7.0	65.7 ± 6.1	51.8 ± 7.1	NC[b]
	11g (R_2 = 4-I-C_6H_5)	57.1 ± 7.4	95.5 ± 2.0	68.9 ± 4.4	11.04 ± 1.9
 13a-e	**13a** (R_3 = CH_3)	78.1 ± 3.3	50.6 ± 3.4	87.14 ± 5.1	12.75 ± 5.8
	13b (R_3 = C_2H_5)	59.7 ± 2.4	39.5 ± 2.5	71.5 ± 1.9	12.6 ± 2.2
	13c (R_3 = C_3H_7)	82.7 ± 4.9	66.5 ± 3.0	98.4 ± 1.5	29.72 ± 2.6
	13d (R_3 = C_4H_9)	NC[b]	16.9 ± 2.4	17.2 ± 4.9	18.31 ± 0.8
	13e (R_3 = C_5H_{11})	71.8 ± 4.7	68.9 ± 1.8	99.3 ± 0.4	15.3 ± 2.5
 14a-e	**14a** (R_4 = C_3H_5)	19.7 ± 4.1	15.6 ± 3.7	8.8 ± 1.5	14.85 ± 3.8
	14b (R_4 = C_4H_7)	97.3 ± 2.6	90.9 ± 3.4	100	66.45 ± 3.2
	14c (R_4 = C_5H_9)	52.8 ± 2.9	64.9 ± 7.7	76.4 ± 4.3	18.3 ± 3.4
	14d (R_4 = C_6H_{11})	68.7 ± 2.2	76.2 ± 6.2	85.2 ± 2	14.04 ± 3.3
	14e (R_4 = C_7H_{13})	72.0 ± 9.4	87.8 ± 5.7	78.9 ± 5.6	13.47 ± 2.2

[a] These values were obtained with a concentration of 25 μM.
[b] NC = No cytotoxic.

Table 2
IC_{50} values for the most active compounds in SKLU-1 cancer cell line.

Compound	IC_{50} (μM)
10a	18.4 ± 0.5
10d	13.1 ± 1.2
11d	12.8 ± 0.5
13e	24.2 ± 2.1
14b	17.4 ± 0.5
Etoposide	4.1 ± 0.6

with the ester at C-3, the number of enones did not seem to be relevant for the activity on the three cancer cell lines, but derivatives with an α,β-unsaturated carbonyl at C-3 (**11c-g**) were less toxic for the non-cancerous cell line than the compounds with α,β-unsaturated dicarbonyls at C-3 and C-6 (**10a-d**). Regarding the aliphatic ester derivatives, compound **13e** just had an IC_{50} of 24.2 ± 2.1 μM. These results will enable us to improve the design of new cytotoxic molecules, knowing that the best position for a lipophilic moiety is at C-21 and that the toxicity on noncancerous cell lines could be determine by the number of conjugations.

In conclusions, the synthesis of three series of steroids with one or more conjugated carbonyls and an ester moiety at C-21 or C-3 were developed. These preliminary biological results showed that the number of conjugated carbonyl groups of these series of compounds did not determine the cytotoxicity on the three cancer cell lines studied, but molecules with just one α,β-unsaturated carbonyl at C-3 (**11c-g**) were less toxic for the noncancerous cell line than the compounds with two α,β-unsaturated carbonyls at C-3 and C-6 (**10a-d**). The final derivatives were evaluated in a panel of three cancer cell lines, the results showed that the compounds with a 4-fluorinated benzoic acid ester moiety (**10d** and **11d**) were the most active steroids against lung cancer cell line with an IC_{50} of 13.1 ± 1.2 and 12.8 ± 0.5 μM respectively and were less toxic than etoposide on the healthy cell line.

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