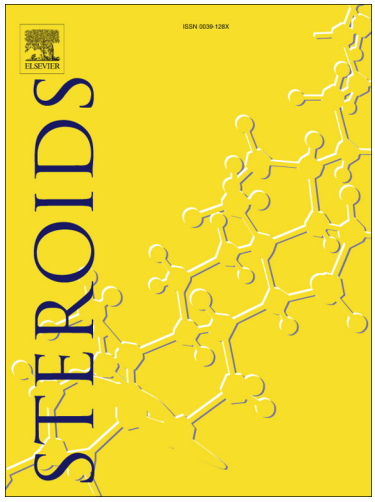

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Design, synthesis and antiproliferative effect of 17β-amide derivatives of 2-methoxyestradiol and their studies on pharmacokinetics

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Abstract

A series of 17β-amide-2-methoxyestradiol compounds were synthesized with an aim to enhance the antiproliferative effect of 2-methoxyestradiol. The antiproliferative activity of 2-methoxyestradiol analogs against human cancer cells was investigated. 2-methoxy-3-benzyloxy-17β-chloroacetamide-1,3,5(10)-triene (**5e**) and 2-methoxy-3-hydroxy-17β-butyramide-1,3,5(10)-triene (**6c**) had comparable or better antitumor activity than 2-methoxyestradiol. The elimination half-life of **6c** ($t_{1/2\beta}$ = 240.93 min) is ten times longer than 2-ME and the area under the curve was seven times ($AUC_{0\text{-}tmin}$ =2068.20 ± 315.74 $\mu g \cdot ml^{-1} \cdot min$) higher than 2-ME, respectively. Whereas **5e** had

similar pharmacokinetic behavior with 2-ME ($t_{1/2\beta}$ = 22.28 min) with a $t_{1/2\beta}$ of 29.5 min. **6c** had higher blood concentration, longer actuation duration and better suppression rate against S180 mouse ascites tumor than 2-methoxyestradiol.

Keywords: 2-methoxyestradiol, analogs, antiproliferative effect, pharmacokinetics

1. Introduction

2-methoxyestradiol (2-ME), a metabolite of estrogen, is an investigational anticancer agent toward a wide range of tumor models such as breast cancer, prostate cancer, ovarian cancer, lung cancer and colon cancer, and inhibits tumor growth at doses showing no clinical signs of toxicity [1, 2]. It also possesses antiangiogenic activity through a direct apoptotic effect on endothelial cells [3]. Main anticancer molecular mechanisms of 2-ME are due to the binding to the colchicine binding site of tubulin, inhibiting polymerization and causing mitotic arrest in G2/M-phase [4]. 2-ME also inhibits HIF-1a translation and its nuclear translocation, and inhibits angiogenesis caused by either bFGF or VEGF [5].

Since the antiproliferative effect and antiangiogenic activity of 2-ME [6-8] were discovered, the synthetic methodology of preparing 2-ME has been a hot focus, and several synthetic routes have been improved for higher yields and fewer steps [9, 10]. However, due to its low water solubility, low bioavailability [11] and its extensive metabolism [12, 13], the clinical application of 2-ME was limited.

Fig. 1 here.

Except for improving formulations [14], the synthesis of active 2-ME analogs or prodrugs has attracted more attentions [15-18]. There are numerous analogs of 2-ME

exhibiting potential anticancer activity even better than 2-ME, and some analogs have been in preclinical or in Phase I clinical trials such as STX140 and ENMD-1198 (**Fig. 1**) [19-21]. Structural modification of 2-ME mainly focuses on ring A at C-2 or C-3 position and ring D at C-16 or C-17 position and others [22-25]. Structure-activity relationships (SAR) of 2-ME analogs have made progress [1, 16, 26].

Fig.2 here.

SAR studies showed the substituent at the C-2 position associated with antiproliferative activity and antitubulin polymerization. Cushman's group concluded that the optimum 2-substituent for cytotoxic activity appeared to be an unbranched chain containing three atoms such as 2-ethoxyestradiol, or 2-(1'-propynyl) estradiol, or 2-(1'-propenyl) estradiol exhibiting the best antiproliferative and antitubulin profile [27]. Potter's group reported that 3-O-sulfamate-2-ethylestradiol and estrone exhibited high antiproliferative activity *in vitro* against a wide range of tumor cells compared to the 2-ethylestradiol [26]. In addition, when the oxygen atom at C-2 position was replaced by bioisosteres NH or S, they displayed slight reduction in activity.

Further SAR studies demonstrated the substituents at C-3 and C-17 position were also associated with bioavailability apart from antitumor activity. Treston's group found that when the 3-OH group of 2-ME was replaced by hydrogen donor substituents, such as 3-NHCOH, 3-NHCN, 3-$NHCONH_2$, it showed much better antiproliferative activities and increased metabolic stability, but the introduction of 3-NH_2 led to substantial loss of activity [28]. And the result indicated that when the C-17 position of 2-ME was exocyclic double bond. The antiproliferative activity of 3-

$CONH_2$ substituent was greater than 3-NHCOH.

Moreover, a series of 2-ME analogs with polar, ionizable, alkyl, endocyclic, or exocyclic olefins at the C-17 position were prepared. Treston investigated that when C-17 position was replaced by amine and carboxamide, it had little effect on antiproliferative activity compared to 2-ME (Fig 2. A1, A2 and A3) [25]. But it reduced or prevented metabolism to make 17-OH deoxygen, or induce endocyclic or exocyclic olefins on the D-ring. ENMD-1198 was one example which has been tested in Phase I clinical trial [23].

Potter [16] showed that optimal activity resulted from the combined presence of a C-2 XMe group (X=O, CH_2 or S), 3-O-sulfamate, and an H-bond acceptor around the C-17 position. They demonstrated that the C-17 oxygen was not necessary for the activity. While the oxygen linker between C-17 and the SO_2 group was replaced with a CH_2 group or a NH group, H-bond acceptor group (O) and an electronically neutral group (CH_2) were tolerated, an H-bond donating group (NH) led to a marked decrease in antiproliferative activity (**Fig.2. A4** and **A5**). However, to the best of our knowledge, there has been no structure-activity relationships established for 17-acylamino (-NHCOR) substituted 2-ME analogs. In order to further explore the SAR of 2-ME and enrich the structure types of the 2-ME analogs, we thus set out to synthesize C-17-acylamino substituent analogs of 2-ME in the hope of discovering additional antitumor compounds with improved activity and metabolic stability.

In this work, 2-methoxy-3-*O*-benzyl-17β-aminoestra-1,3,5(10)-triene was synthesized in two steps from 2-methoxy-3-*O*-benzylestrone as described by Gonschior et al [29], and then reacted with various acyl chlorides, followed by hydrogenolysis to form 17β-acylamide derivatives of 2-methoxyestradiol. The effect of different substitutions such as alkyl, aryl, halogen, and sulfonamide at the C-17

amide chain was investigated. The analogs were further evaluated *in vitro* antiproliferation activity against tumor cells, and synthetic compounds **5e**, **5n**, and **6c** had comparable or better activity than 2-ME. Analogs with acceptable in vitro profiles were further assessed for pharmacokinetic (PK) properties *in vivo*.

2. Experimental

2.1. Chemistry

NMR spectra were recorded on a Bruker Avance DPX-400 MHz spectrometer for ^{1}H NMR, and 101 MHz for ^{13}C NMR. High-resolution mass spectra (HRMS) were recorded on Esquire 3000 mass spectrometer by electrospray ionization (ESI). X-ray single crystal diffraction data was collected on an Oxford Xcalibur, Gemini, Eos CCD diffractometer. Estradiol was purchased from Wuhan Kai Lun Chemical New Material Company. All solvents were analytical grade unless stated otherwise.

*2.1.1. General procedure for the preparation of 2-Methoxy-3-benzyloxy-17-carbonyl-1,3,5(10)-triene (**2**).*

Compound **1** (1.75 g, 4.5 mmol) was dissolved in toluene (60 mL) in a round-bottom flask. After 30 min reflux, cyclohexanone (18.7 mL, 22.5 mmol) was added and the mixture refluxed for 1 h, then aluminum isopropoxide (4.627 g, 22.5 mmol) was added and heated at 140 ^{o}C for 24 h. An aqueous solution of sodium bicarbonate (100 mL) was added into the reaction mixture when the reaction mixture was cooled to room temperature. After stirring for 1 h, the reaction mixture was extracted with CH_2Cl_2 three times. The combined organic layers were washed with an aqueous solution of sodium bicarbonate, saturated sodium chloride successively and dried over (Na_2SO_4). The organic layer was filtered and concentrated under reduced pressure to a thick colorless oil, then 250 mL petroleum ether was added for recrystallization to afford pure product **2** as a white solid (1.42 g, 82%), mp 155-156 ^{o}C; ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.47 (d, J = 7.1 Hz, 2H), 7.43 – 7.36 (m, 2H), 7.35 – 7.29 (m, 1H), 6.87 (s, 1H), 6.67 (s, 1H), 5.13 (s, 2H), 3.89 (s, 3H), 2.91 – 2.74 (m, 2H), 2.53 (dd, J = 18.9, 8.5 Hz, 1H), 2.46 – 2.37 (m, 1H), 2.34 – 2.23 (m, 1H), 2.23 – 1.95 (m, 4H), 1.71 – 1.44 (m, 6H), 0.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.69, 146.45, 137.39, 132.31, 128.60, 128.52, 127.76, 127.30, 114.61, 109.63, 77.39, 77.08, 76.76, 71.10, 56.28, 50.39, 48.04, 44.29, 38.30, 35.91, 31.64, 29.04, 26.64, 26.13, 21.59, 13.91.

*2.1.2. General procedure for the preparation of 2-methoxy-3-benzyloxy-17-oxime-1,3,5(10)-triene (**3**).*

Hydroxylamine hydrochloride (0.178 g, 2.564 mmol) was added into a solution of compound **2** (0.5 g, 1.282 mmol) and pyridine (5 ml). The reaction mixture was allowed to stir at 60 ^{o}C for 1 h. TLC showed that the reaction was complete. The reaction mixture was cooled to 0 ^{o}C and substantial ice water was poured into the mixture. The reaction mixture was filtered and the filter cake was washed with water, dried in vacuum to afford the crude product. The crude product was purified by silica gel chromatography to give product **3** as a white solid (0.487 g, 94%), mp 157-159 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 7.49 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.3 Hz, 2H), 7.34 (t, J = 7.2 Hz, 1H), 6.89 (s, 1H), 6.68 (s, 1H), 5.15 (s, 2H), 3.91 (s, 3H), 2.91 – 2.75 (m, 2H), 2.70 – 2.58 (m, 2H), 2.45 – 2.37 (m, 1H), 2.33 (dd, J = 14.3, 7.3 Hz, 1H), 2.16 – 2.08 (m, 1H), 2.02 – 1.93 (m, 2H), 1.78 – 1.56 (m, 3H), 1.50 – 1.34 (m, 3H), 1.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.13, 147.68, 146.44, 137.44, 132.60, 128.68, 128.51, 127.74, 127.31, 114.67, 109.73, 77.38, 77.06, 76.74, 71.13, 56.34, 52.89, 44.33, 44.31, 38.09, 34.11, 29.07, 27.35, 26.37, 25.14, 22.93, 17.26.

*2.1.3. General procedure for the preparation of 2-methoxy-3-benzyloxy-17β-amino-1,3,5(10)-triene (**4**)*

To a solution of compound **3** (0.81 g, 2 mmol) in a mixture of MeOH (20 mL) and

THF (5 mL) was added MoO_3 (0.63 g, 4.4 mmol) at 0 ^oC and then $NaBH_4$ was added portion-wise till the reaction was complete confirmed by TLC. 10% solution of potassium hydroxide (5 mL) was added at 0 ^oC and stirred overnight. The suspension was filtered through a pad of silica gel and filter cake was washed with MeOH. The combined filtrates were concentrated and dissolved with CH_2Cl_2, then washed with water and saturated sodium chloride successively. The organic layers were dried over ($MgSO_4$) and concentrated to colorless oil. The crude product was purified by column chromatography on silica gel to give product **4** as a white solid (0.635 g, 81 %), mp 60-62 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 7.1 Hz, 2H), 7.38 (t, J = 7.3 Hz, 2H), 7.35 – 7.29 (m, 1H), 6.86 (d, J = 12.7 Hz, 1H), 6.69 – 6.60 (m, 1H), 5.11 (d, J = 13.6 Hz, 2H), 3.92 – 3.80 (m, 3H), 3.18 (dd, J = 37.0, 28.3 Hz, 1H), 2.88 – 2.63 (m, 2H), 2.40 – 2.15 (m, 4H), 2.00 – 1.71 (m, 3H), 1.62 – 1.21 (m, 6H), 1.07 – 0.84 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.65, 146.47, 137.42, 132.45, 128.69, 128.50, 127.74, 127.31, 114.68, 109.79, 77.37, 77.05, 76.74, 71.13, 61.14, 56.35, 51.69, 43.92, 42.82, 38.52, 36.54, 28.99, 27.46, 26.22, 23.61, 12.21.

*2.1.4. General procedure for the preparation of analogs 2-methoxy-3-benzyloxy-17β-amide-1,3,5(10)-triene series (**5a - 5n**).*

To a solution of compound **4** (1 mmol) in CH_2Cl_2 (10 mL) was added NEt_3 (2 mmol) at 0 ^oC. Acid chloride (1.3 mmol) was added dropwise at 0 ^oC. After stirring 30 mins, the reaction was done confirmed by TLC. The reaction mixture was extracted with water and CH_2Cl_2. The separated organic layer was washed with water and saturated sodium chloride successively, and dried over ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was recrystallized from acetone and petroleum ether to give products **5a - 5n**.

*2.1.4.1. 2-methoxy-3-benzyloxy-17β-acetamide-1,3,5(10)-triene (**5a**)*

5a, given as a white solid; Yield: 84%; mp 197-198 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ7.47 (d, J = 7.3 Hz, 2H), 7.38 (t, J = 7.3 Hz, 2H), 7.31 (dd, J = 12.3, 5.0 Hz, 1H), 6.86 (s, 1H), 6.64 (s, 1H), 5.35 (t, J = 9.0 Hz, 1H), 5.13 (s, 2H), 4.07 – 3.97 (m, 1H), 3.89 (s, 3H), 2.89 – 2.63 (m, 2H), 2.33 – 2.22 (m, 2H), 2.18 (dd, J = 12.0, 6.3 Hz, 1H), 2.03 (s, 3H), 1.92 – 1.76 (m, 3H), 1.46 – 1.29 (m, 6H), 0.93 – 0.85 (m, 1H), 0.75 (s, 3H).

*2.1.4.2. 2-methoxy-3-benzyloxy-17β-propionamide-1,3,5(10)-triene (**5b**)*

5b, given as a white solid; Yield: 94%; mp 159-160 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.3 Hz, 2H), 7.39 (dd, *J* = 10.0, 4.7 Hz, 2H), 7.32 (dd, *J* = 8.4, 6.2 Hz, 1H), 6.86 (s, 1H), 6.64 (s, 1H), 5.32 (d, *J* = 9.0 Hz, 1H), 5.13 (s, 2H), 4.03 (dd, *J* = 17.7, 8.8 Hz, 1H), 3.89 (s, 3H), 2.88 – 2.67 (m, 2H), 2.33 – 2.16 (m, 5H), 1.92 – 1.82 (m, 2H), 1.56 – 1.26 (m, 8H), 1.20 (t, *J* = 7.6 Hz, 3H), 0.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.66, 147.64, 146.38, 137.49, 132.98, 128.81, 128.48, 127.70, 127.30, 114.73, 109.92, 77.36, 77.04, 76.72, 71.16, 58.67, 56.39, 51.60, 44.14, 43.32, 38.93, 37.02, 30.01, 29.16, 28.84, 27.43, 26.44, 23.30, 12.12, 10.10.

*2.1.4.3. 2-methoxy-3-benzyloxy-17β-butyramide-1,3,5(10)-triene (**5c**)*

5c, given as a white solid; Yield: 75%; mp 136-137 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 7.2 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 6.86 (s, 1H), 6.64 (s, 1H), 5.32 (d, J = 9.1 Hz, 1H), 5.12 (s, 2H), 4.09 – 3.99 (m, 1H), 3.88 (s, 3H), 2.84 – 2.70 (m, 2H), 2.27 (dd, J = 17.1, 9.5 Hz, 2H), 2.23 – 2.16 (m, 3H), 1.92 – 1.69 (m, 5H), 1.51 (dd, J = 15.5, 11.3 Hz, 2H), 1.43 – 1.30 (m, 5H), 0.99 (t, J = 7.4 Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.85, 147.64, 146.38, 137.49, 132.99, 128.81, 128.48, 127.71, 127.31, 114.73, 109.91, 77.36, 77.05, 76.73, 71.16, 58.65, 56.38, 51.58, 44.14, 43.35, 39.06, 38.93, 37.05, 29.15, 28.82, 27.43, 26.45, 23.31, 19.36, 13.77, 12.15.

*2.1.4.4. 2-methoxy-3-benzyloxy-17β-pivaloylamide-1,3,5(10)-triene (**5d**)*

5d, given as a white solid; Yield: 85%; mp 140-141 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.2 Hz, 2H), 7.39 (dd, *J* = 10.0, 4.7 Hz, 2H), 7.35 – 7.29 (m, 1H), 6.86 (s, 1H), 6.65 (s, 1H), 5.50 (d, *J* = 8.8 Hz, 1H), 5.13 (s, 2H), 4.02 (q, *J* = 8.9 Hz, 1H), 3.89 (s, 3H), 2.85 – 2.67 (m, 2H), 2.33 – 2.12 (m, 3H), 1.85 (ddd, *J* = 11.5, 9.5, 2.8 Hz, 3H), 1.57 – 1.31 (m, 7H), 1.24 (s, 9H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.36, 147.63, 146.38, 137.50, 133.00, 128.82, 128.48, 127.70, 127.30, 114.71, 109.93, 77.36, 77.04, 76.72, 71.15, 58.49, 56.39, 51.60, 44.12, 43.55, 38.94, 38.80, 37.16, 29.16, 28.81, 27.74, 27.44, 26.46, 23.34, 12.08.TOF MS ES(+)(m/z): calculated for $C_{31}H_{42}NO_3$ ($[M+H]^+$) 476.3165; found 476.3168.

*2.1.4.5. 2-methoxy-3-benzyloxy-17β-chloroacetamide-1,3,5(10)-triene (**5e**)*

5e, given as a white solid; Yield: 64%; mp 169-170 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.8 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.29 (m, 1H), 6.86 (s, 1H), 6.65 (s, 1H), 6.53 (d, *J* = 8.9 Hz, 1H), 5.13 (s, 2H), 4.09 (d, *J* = 15.5 Hz, 2H), 4.05 – 3.96 (m, 1H), 3.89 (s, 3H), 2.90 – 2.67 (m, 2H), 2.38 – 2.17 (m, 3H), 1.96 – 1.77 (m, 3H), 1.55 – 1.31 (m, 7H), 0.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.64, 147.60, 146.37, 137.44, 132.73, 128.74, 128.51, 127.73, 127.31, 114.61, 109.77, 77.38, 77.06, 76.75, 71.09, 59.12, 56.36, 51.60, 44.11, 43.55, 42.93, 38.85, 36.88, 29.14, 28.61, 27.43, 26.37, 23.35, 12.06.TOF MS ES(+)(m/z): calculated for $C_{28}H_{35}ClNO_3$ ($[M+H]^+$) 468.2305; found 468.2335.

*2.1.4.6. 2-methoxy-3-benzyloxy-17β-(3'-chloro) propionamide-1,3,5(10)-triene (**5f**)*

5f, given as a yellow solid; Yield: 80%; mp 103-104 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 1H), 6.86 (s, 1H), 6.65 (d, *J* = 8.6 Hz, 1H), 5.53 (t, *J* = 11.3 Hz, 1H), 5.14 (d, *J* = 7.5 Hz, 2H), 4.19 – 4.00 (m, 1H), 3.92 – 3.80 (m, 5H), 2.89 – 2.50 (m, 4H), 2.33 – 2.13 (m,

3H), 1.92 – 1.85 (m, 2H), 1.84 – 1.77 (m, 1H), 1.38 (dd, *J* = 18.6, 15.3 Hz, 5H), 1.02 – 0.82 (m, 2H), 0.77 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.39, 147.57, 146.32, 137.45, 132.88, 128.74, 128.50, 127.73, 127.31, 114.59, 109.77, 77.38, 77.06, 76.74, 71.10, 59.06, 56.33, 51.53, 44.11, 43.51, 40.56, 40.02, 38.89, 36.97, 29.15, 28.66, 27.44, 26.43, 23.33, 12.15.TOF MS ES(+)(m/z): calculated for $C_{29}H_{37}ClNO_3$ ([M +H]$^+$) 482.2462; found 482.2461.

*2.1.4.7. 2-methoxy-3-benzyloxy-17β-(3'-fluoro) propionamide-1,3,5(10)-triene (**5g**)*

5g, given as a white solid; Yield: 75%; mp 108-109 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.3 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 1H), 6.86 (s, 1H), 6.64 (s, 1H), 5.58 (s, 1H), 5.13 (s, 2H), 4.08 (dt, *J* = 26.7, 9.0 Hz, 1H), 3.95 – 3.75 (m, 5H), 2.88 – 2.56 (m, 4H), 2.33 – 2.14 (m, 3H), 1.88 (dd, *J* = 7.3, 3.8 Hz, 2H), 1.84 – 1.77 (m, 1H), 1.56 – 1.31 (m, 7H), 0.77 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.41, 147.57, 146.32, 137.45, 132.88, 128.75, 128.51, 127.74, 127.32, 114.59, 109.78, 77.39, 77.08, 76.76, 71.10, 59.06, 56.34, 51.53, 44.11, 43.51, 40.57, 40.00, 38.89, 36.98, 29.15, 28.64, 27.44, 26.43, 23.33, 12.15.

*2.1.4.8. 2-methoxy-3-benzyloxy-17β-benzamide-1,3,5(10)-triene (**5h**)*

5h, given as a white solid; Yield: 95%; mp 152-154 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.74 (m, 2H), 7.53 (ddd, *J* = 6.2, 3.6, 1.3 Hz, 1H), 7.50 – 7.44 (m, 4H), 7.39 (dd, *J* = 10.0, 4.7 Hz, 2H), 7.35 – 7.29 (m, 1H), 6.97 – 6.77 (m, 1H), 6.66 (s, 1H), 6.06 (d, *J* = 8.8 Hz, 1H), 5.14 (s, 2H), 4.37 – 4.11 (m, 1H), 3.85 (d, *J* = 30.6 Hz, 3H), 2.92 – 2.67 (m, 2H), 2.45 – 2.21 (m, 3H), 1.89 (ddd, *J* = 23.9, 10.9, 3.9 Hz, 3H), 1.67 – 1.22 (m, 8H), 0.88 – 0.82 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.47, 147.60, 146.35, 137.47, 135.04, 132.90, 131.38, 128.77, 128.62, 128.51, 127.73, 127.32, 126.83, 114.63, 109.81, 77.39, 77.08, 76.76, 71.11, 59.25, 56.37, 51.66, 44.15, 43.78, 38.95, 37.12, 29.19, 28.93, 27.47, 26.47, 23.43, 12.31.

*2.1.4.9. 2-methoxy-3-benzyloxy-17β-(4'-fluoro) benzamide-1,3,5(10)-triene (**5i**)*

5i, given as a white solid; Yield: 91%; mp 163-164 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.76 (m, 2H), 7.47 (d, J = 7.2 Hz, 2H), 7.42 – 7.36 (m, 2H), 7.35 – 7.29 (m, 1H), 7.18 – 7.10 (m, 2H), 6.87 (s, 1H), 6.65 (s, 1H), 5.95 (d, J = 8.9 Hz, 1H), 5.13 (s, 2H), 4.30 – 4.14 (m, 1H), 3.89 (s, 3H), 2.89 – 2.67 (m, 2H), 2.36 – 2.22 (m, 3H), 1.97 – 1.78 (m, 3H), 1.60 – 1.32 (m, 7H), 0.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.42, 147.65, 146.40, 137.47, 132.88, 129.16, 129.07, 128.80, 128.50, 127.73, 127.32, 115.72, 115.50, 114.72, 109.91, 77.37, 77.05, 76.73, 71.15, 59.36, 56.40, 51.66, 44.14, 43.77, 38.95, 37.13, 29.16, 28.93, 27.45, 26.45, 23.42, 12.32.TOF MS ES(+)(m/z): calculated for $C_{29}H_{37}FNO_3$ ($[M+H]^+$) 514.2757; found 514.2771.

*2.1.4.10. 2-methoxy-3-benzyloxy-17β-phenylacetamide-1,3,5(10)-triene (**5j**)*

5j, given as a white solid; Yield: 86%; mp 164-166 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.2 Hz, 2H), 7.43 – 7.29 (m, 8H), 6.84 (s, 1H), 6.63 (s, 1H), 5.24 (d, *J* = 9.1 Hz, 1H), 5.12 (s, 2H), 3.99 (q, *J* = 9.0 Hz, 1H), 3.88 (s, 3H), 3.68 – 3.56 (m, 2H), 2.74 (q, *J* = 10.2 Hz, 2H), 2.26 (dd, *J* = 13.2, 5.4 Hz, 2H), 2.12 (dt, *J* = 12.6, 6.7 Hz, 1H), 1.90 – 1.71 (m, 3H), 1.49 – 1.17 (m, 7H), 0.50 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.90, 147.64, 146.39, 137.48, 135.22, 132.92, 129.41, 129.07, 128.79, 128.48, 127.71, 127.40, 127.31, 114.72, 109.89, 77.38, 77.06, 76.74, 71.15, 58.75, 56.37, 51.54, 44.10, 43.39, 38.83, 36.94, 29.11, 28.63, 27.41, 26.40, 23.27, 11.76.TOF MS ES(+)(m/z): calculated for $C_{34}H_{40}NO_3$ ($[M+H]^+$) 510.3008; found 510.3050.

*2.1.4.11. 2-methoxy-3-benzyloxy-17β-(1',2',3',4'-tetrafluoro) phenylacetamide-1,3,5(10)-triene (**5k**)*

5k, given as a white solid; Yield: 90%; mp 163-165 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.72 (m, 1H), 7.47 (d, J = 7.2 Hz, 2H), 7.39 (dd, J = 10.0, 4.6 Hz, 2H), 7.32 (dd, J = 8.5, 6.0 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.65 (s, 1H), 6.55 (dd, J = 11.5, 9.1

Hz, 1H), 5.13 (s, 2H), 4.21 (tt, J = 8.9, 4.3 Hz, 1H), 3.89 (s, 3H), 2.88 – 2.70 (m, 2H), 2.34 (dd, J = 15.6, 8.4 Hz, 3H), 1.98 – 1.80 (m, 3H), 1.67 – 1.32 (m, 8H), 0.86 (d, J = 6.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.25, 147.61, 146.39, 137.43, 132.69, 128.75, 128.51, 127.74, 127.31, 114.62, 113.07, 112.85, 109.79, 77.37, 77.05, 76.73, 71.10, 59.85, 56.37, 51.60, 44.11, 43.70, 38.89, 37.06, 29.14, 28.93, 27.45, 26.40, 23.45, 12.28.

*2.1.4.12. 2-methoxy-3-benzyloxy-17β-methanesulfonamide-1,3,5(10)-triene (**5l**)*

5l, given as a white solid; Yield: 98%; mp 200-202 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 1H), 6.86 (s, 1H), 6.65 (s, 1H), 5.13 (s, 2H), 4.42 (d, *J* = 9.5 Hz, 1H), 3.89 (s, 3H), 3.51 (d, *J* = 4.9 Hz, 2H), 3.37 (q, *J* = 9.2 Hz, 1H), 3.01 (s, 3H), 2.78 (dt, *J* = 17.0, 9.3 Hz, 2H), 2.39 – 2.17 (m, 3H), 2.06 – 1.93 (m, 1H), 1.92 – 1.74 (m, 2H), 1.46 – 1.26 (m, 5H), 0.77 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 147.61, 146.39, 137.41, 132.58, 128.72, 128.51, 127.75, 127.31, 114.61, 109.73, 77.38, 77.06, 76.75, 71.09, 63.53, 56.34, 51.21, 50.90, 44.14, 42.88, 41.65, 38.83, 36.66, 30.09, 29.09, 27.33, 26.28, 23.21, 11.90.

*2.1.4.13. 2-methoxy-3-benzyloxy-17β-p-toluenesulfonamide-1,3,5(10)-triene (**5m**)*

5m, given as a white solid; Yield: 93%; mp 147-148 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.35 – 7.29 (m, 3H), 6.83 (s, 1H), 6.63 (s, 1H), 5.12 (s, 2H), 4.66 (d, *J* = 9.2 Hz, 1H), 3.88 (s, 3H), 3.18 (q, *J* = 8.9 Hz, 1H), 2.83 – 2.61 (m, 2H), 2.47 (s, 3H), 2.33 – 2.11 (m, 2H), 1.96 – 1.76 (m, 3H), 1.66 (s, 1H), 1.52 – 1.11 (m, 7H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.59, 146.35, 143.23, 138.18, 137.42, 132.68, 129.64, 128.68, 128.50, 127.73, 127.29, 127.15, 114.58, 109.70, 77.38, 77.07, 76.75, 71.08, 63.35, 56.32, 51.12, 44.12, 42.93, 38.77, 36.38, 29.49, 29.07, 27.32, 26.25, 23.16, 21.60, 11.89.

*2.1.4.14. 2-methoxy-3-benzyloxy-17β-niacinamide-1,3,5(10)-triene (**5n**)*

5n, given as a white solid; Yield: 88%; mp 169-170 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, J = 2.0 Hz, 1H), 8.75 (dd, J = 4.8, 1.5 Hz, 1H), 8.14 (dt, J = 7.9, 1.9 Hz, 1H), 7.47 (d, J = 7.3 Hz, 2H), 7.40 (dt, J = 14.5, 6.2 Hz, 3H), 7.31 (t, J = 7.3 Hz, 1H), 6.86 (s, 1H), 6.65 (s, 1H), 6.11 (d, J = 8.8 Hz, 1H), 5.13 (s, 2H), 4.24 (q, J = 9.0 Hz, 1H), 3.88 (d, J = 7.7 Hz, 3H), 2.88 – 2.68 (m, 2H), 2.39 – 2.23 (m, 3H), 1.98 – 1.78 (m, 4H), 1.65 – 1.31 (m, 7H), 0.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.57, 152.21, 147.70, 147.63, 146.40, 137.45, 135.09, 132.79, 130.66, 128.76, 128.50, 127.73, 127.31, 123.57, 114.68, 109.85, 77.38, 77.06, 76.75, 71.13, 59.44, 56.38, 51.67, 44.13, 43.83, 38.92, 37.13, 29.15, 28.89, 27.45, 26.44, 23.42, 12.35.TOF MS ES(+)(m/z): calculated for $C_{32}H_{37}N_2O_3$ ($[M+H]^+$) 497.2804; found 497.2805.

*2.1.5. General procedure for the preparation of analogs 2-methoxy-3-hydroxy-17β-amide-1,3,5(10)-triene series (**6a - 6n**).*

To a solution of compound **5a - 5n** (0.3 g) in EtOH (50 mL) was added Pd/C (10 %, 0.03 g). The suspension was degassed and purged with H_2 three times. The mixture was stirred under H_2 (30 psi) at 60 ^{o}C for 4 hours. TLC showed that the reaction was complete. The suspension was filtered through silica gel and filter cake was washed with EtOH. The combined filtrate was concentrated to dryness and then recrystallized from petroleum ether and ethyl acetate to give pure products **6a - 6n**.

*2.1.5.1. 2-methoxy-3-hydroxy-17β-acetamide-1,3,5(10)-triene (**6a**)*

6a, given as a white solid; Yield: 60%; mp 253-254 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (s, 1H), 5.55 (s, 1H), 5.38 (d, J = 9.0 Hz, 1H), 4.01 (d, J = 8.9 Hz, 1H), 3.88 (s, 3H), 2.87 – 2.70 (m, 2H), 2.22 (ddd, J = 17.9, 10.4, 4.5 Hz, 3H), 2.11 – 1.97 (m, 3H), 1.94 – 1.75 (m, 3H), 1.60 – 1.21 (m, 7H), 0.76 (d, J = 9.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.04, 144.63, 143.49, 131.68, 129.42, 114.63, 108.15,

77.38, 77.06, 76.75, 58.90, 56.11, 51.56, 44.13, 43.27, 38.96, 36.94, 29.01, 28.76, 27.41, 26.55, 23.65, 23.28, 12.14.

*2.1.5.2. 2-methoxy-3-hydroxy-17β-propionamide-1,3,5(10)-triene (**6b**)*

6b, given as a white solid; Yield: 54%; mp 171-172 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (s, 1H), 5.61 (d, J = 8.7 Hz, 1H), 5.35 (t, J = 11.1 Hz, 1H), 4.08 – 3.97 (m, 1H), 3.88 (s, 3H), 2.83 – 2.72 (m, 2H), 2.30 – 2.13 (m, 6H), 1.92 – 1.73 (m, 4H), 1.55 – 1.29 (m, 7H), 1.20 (t, J = 7.6 Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.76, 144.63, 143.49, 131.71, 129.42, 114.63, 108.15, 77.38, 77.07, 76.75, 58.67, 56.11, 51.57, 44.13, 43.34, 38.97, 37.00, 30.02, 29.02, 28.80, 27.42, 26.56, 23.30, 12.12, 10.14.

*2.1.5.3. 2-methoxy-3-hydroxy-17β-butyramide-1,3,5(10)-triene (**6c**)*

6c, given as a white solid; Yield: 75%; mp 174-175 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (s, 1H), 5.53 (s, 1H), 5.33 (d, *J* = 9.0 Hz, 1H), 4.09 – 3.99 (m, 1H), 3.88 (s, 3H), 2.85 – 2.74 (m, 2H), 2.29 – 2.16 (m, 5H), 1.93 – 1.77 (m, 3H), 1.69 (dd, *J*= 7.4, 2.0 Hz, 1H), 1.57 – 1.27 (m, 8H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.91, 144.64, 143.52, 131.73, 129.44, 114.63, 108.18, 77.36, 77.04, 76.73, 58.67, 56.11, 51.59, 44.13, 43.36, 39.05, 38.99, 37.04, 29.00, 28.80, 27.41, 26.58, 23.31, 19.36, 13.76, 12.14.TOF MS ES(+)(m/z): calculated for $C_{23}H_{34}NO_3$([M +H]$^{+}$) 372.2539; found 372.2538.

*2.1.5.4. 2-methoxy-3-hydroxy-17β-pivaloylamide-1,3,5(10)-triene (**6d**)*

6d, given as a brown solid; Yield: 60%; mp 127-129 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (s, 1H), 5.61 – 5.48 (m, 2H), 4.08 – 3.95 (m, 1H), 3.88 (s, 3H), 2.89 – 2.70 (m, 2H), 2.34 – 2.12 (m, 3H), 1.93 – 1.85 (m, 1H), 1.83 – 1.77 (m, 2H), 1.46 – 1.29 (m, 6H), 1.24 (s, 9H), 1.00 – 0.81 (m, 1H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.43, 144.61, 143.49, 131.74, 129.43, 114.63, 108.14, 77.38,

77.06, 76.75, 58.49, 56.10, 51.57, 44.11, 43.56, 38.99, 38.81, 37.14, 29.02, 28.79, 27.74, 27.42, 26.59, 23.33, 12.08.

*2.1.5.5. 2-methoxy-3-hydroxy-17β-chloroacetamide-1,3,5(10)-triene (**6e**)*

6e, given as a white solid; Yield: 69%; mp 226-228 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (d, J = 12.2 Hz, 1H), 6.70 – 6.60 (m, 1H), 5.66 (d, J = 3.0 Hz, 1H), 5.45 (d, J = 8.8 Hz, 1H), 4.07 – 3.95 (m, 1H), 3.87 (d, J = 6.4 Hz, 3H), 2.87 – 2.71 (m, 2H), 2.33 – 2.12 (m, 4H), 2.03 (s, 3H), 1.93 – 1.73 (m, 4H), 1.55 – 1.25 (m, 8H), 0.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.06, 144.65, 143.51, 131.67, 129.41, 114.65, 108.17, 77.40, 77.08, 76.76, 58.90, 56.11, 51.56, 44.13, 43.27, 38.96, 36.95, 29.01, 28.75, 27.41, 26.55, 23.65, 23.29, 12.15.

*2.1.5.6. 2-methoxy-3-hydroxy-17β-(3'-chloro) propionamide-1,3,5(10)-triene (**6f**)*

6f, given as a white solid; Yield: 84%; mp 149-150 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (s, 1H), 5.56 (dd, *J* = 9.3, 2.0 Hz, 2H), 4.06 (q, *J* = 8.9 Hz, 1H), 3.93 – 3.76 (m, 5H), 2.86 – 2.74 (m, 2H), 2.74 – 2.57 (m, 2H), 2.22 (ddd, *J* = 27.3, 13.0, 5.8 Hz, 3H), 1.94 – 1.84 (m, 2H), 1.84 – 1.78 (m, 1H), 1.52 – 1.30 (m, 7H), 0.76 (d, *J* = 9.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.39, 144.62, 143.48, 131.70, 129.41, 114.62, 108.13, 77.38, 77.07, 76.75, 59.07, 56.10, 51.54, 44.10, 43.52, 40.57, 40.01, 38.95, 36.97, 28.99, 28.64, 27.42, 26.56, 23.33, 12.14.

*2.1.5.7. 2-methoxy-3-hydroxy-17β-(3'-fluoro) propionamide-1,3,5(10)-triene (**6g**)*

6g, given as a yellow solid; Yield: 70%; mp 92-93 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (s, 1H), 5.61 (s, 2H), 4.06 (q, *J* = 8.9 Hz, 1H), 3.95 – 3.77 (m, 5H), 2.86 – 2.74 (m, 2H), 2.74 – 2.58 (m, 2H), 2.32 – 2.14 (m, 3H), 1.86 (dd, *J* = 18.7, 9.5 Hz, 4H), 1.46 – 1.24 (m, 6H), 0.77 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.43, 144.63, 143.48, 131.69, 129.40, 114.64, 108.15, 77.40, 77.08, 76.76, 59.07, 56.10, 51.54, 44.10, 43.53, 40.58, 40.00, 38.95, 36.98, 28.99, 28.62, 27.42, 26.56, 23.33,

12.14.

*2.1.5.8. 2-methoxy-3-hydroxy-17β-benzamide-1,3,5(10)-triene (**6h**)*

6h, given as a white solid; Yield: 89%; mp 125-127 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.69 (m, 2H), 7.61 – 7.39 (m, 3H), 6.81 (s, 1H), 6.67 (s, 1H), 6.07 (d, *J* = 8.9 Hz, 1H), 5.56 (s, 1H), 4.24 (q, *J* = 9.1 Hz, 1H), 3.88 (s, 3H), 2.81 (dd, *J* = 8.3, 4.8 Hz, 2H), 2.41 – 2.22 (m, 3H), 2.00 – 1.80 (m, 3H), 1.65 – 1.29 (m, 7H), 0.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.50, 144.63, 143.50, 135.02, 131.69, 131.39, 129.43, 128.62, 126.83, 114.63, 108.15, 77.38, 77.06, 76.75, 59.26, 56.12, 51.66, 44.15, 43.79, 39.01, 37.11, 29.03, 28.92, 27.44, 26.60, 23.43, 12.30.

*2.1.5.9. 2-methoxy-3-hydroxy-17β-(4'-fluoro) benzamide-1,3,5(10)-triene (**6i**)*

6i, given as a white solid; Yield: 56%; mp 140-141 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.72 (m, 2H), 7.22 – 7.06 (m, 2H), 6.81 (s, 1H), 6.67 (s, 1H), 5.96 (d, *J* = 8.8 Hz, 1H), 5.48 (s, 1H), 4.37 – 4.08 (m, 1H), 4.04 – 3.72 (m, 3H), 2.83 (dd, *J* = 17.9, 11.8 Hz, 2H), 2.50 – 2.15 (m, 3H), 1.98 – 1.78 (m, 3H), 1.62 – 1.27 (m, 7H), 0.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.45, 165.89, 163.39, 144.63, 143.50, 131.65, 131.17, 129.42, 129.17, 129.08, 115.73, 115.51, 114.63, 108.13, 77.38, 77.06, 76.74, 59.35, 56.12, 51.65, 44.13, 43.78, 39.00, 37.11, 29.02, 28.91, 27.43, 26.58, 23.41, 12.32.

*2.1.5.10. 2-methoxy-3-hydroxy-17β-phenylacetamide-1,3,5(10)-triene (**6j**)*

6j, given as a brown solid; Yield: 74%; mp 138-139 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (ddd, J = 16.2, 14.0, 7.5 Hz, 6H), 6.79 (s, 1H), 6.65 (s, 1H), 5.52 (s, 1H), 5.26 (d, J = 9.0 Hz, 1H), 3.99 (dd, J = 18.0, 9.0 Hz, 1H), 3.88 (s, 3H), 3.69 – 3.56 (m, 2H), 2.76 (s, 2H), 2.15 (ddd, J = 26.4, 17.0, 8.1 Hz, 4H), 1.93 – 1.68 (m, 5H), 1.56 – 1.14 (m, 8H), 0.50 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.93, 144.63, 143.52, 135.20, 131.66, 129.41, 129.07, 127.40, 114.63, 108.15, 77.37, 77.05, 76.73,

58.76, 56.10, 51.54, 44.10, 43.40, 38.89, 36.94, 28.96, 28.62, 27.39, 26.53, 23.26, 11.75.

*2.1.5.11. 2-methoxy-3-hydroxy-17β-(1',2',3',4'-tetrafluoro) phenylacetamide-1,3,5(10)-triene (**6k**)*

6k, given as a yellow solid; Yield: 76%; mp 164-165 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (dddd, J = 10.7, 8.7, 6.6, 2.4 Hz, 1H), 6.80 (s, 1H), 6.66 (d, J = 7.5 Hz, 1H), 6.61 – 6.49 (m, 1H), 5.50 (s, 1H), 4.21 (qt, J = 13.1, 6.5 Hz, 1H), 3.88 (s, 3H), 2.86 – 2.72 (m, 2H), 2.31 (ddd, J = 16.5, 10.7, 6.3 Hz, 3H), 1.96 – 1.79 (m, 3H), 1.60 – 1.31 (m, 7H), 0.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.27, 144.61, 143.52, 131.52, 129.41, 114.62, 113.05, 112.85, 108.07, 99.99, 77.36, 77.05, 76.73, 59.85, 56.10, 51.61, 44.11, 43.70, 38.95, 37.06, 28.98, 28.93, 27.43, 26.53, 23.45, 12.27.

*2.1.5.12. 2-methoxy-3-hydroxy-17β-methanesulfonamide-1,3,5(10)-triene (**6l**)*

6l, given as a white solid; Yield: 40%; mp 189-191 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 5.51 (s, 1H), 4.46 (t, *J* = 11.8 Hz, 1H), 3.88 (s, 3H), 3.37 (q, *J* = 9.2 Hz, 1H), 3.00 (d, *J* = 6.2 Hz, 3H), 2.89 – 2.69 (m, 2H), 2.36 – 2.18 (m, 3H), 1.99 (dt, *J* = 12.5, 2.9 Hz, 1H), 1.93 – 1.74 (m, 2H), 1.58 – 1.46 (m, 2H), 1.43 – 1.25 (m, 5H), 0.77 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.63, 143.52, 131.43, 129.38, 114.63, 108.05, 77.39, 77.07, 76.75, 63.53, 56.10, 51.20, 44.13, 42.88, 41.63, 38.89, 36.62, 30.03, 28.94, 27.32, 26.40, 23.22, 11.90.

*2.1.5.13. 2-methoxy-3-hydroxy-17β-p-toluenesulfonamide-1,3,5(10)-triene (**6m**)*

6m, given as a yellow solid; Yield: 77%; mp 164-165 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 6.78 (s, 1H), 6.65 (s, 1H), 5.46 (s, 1H), 4.51 (s, 1H), 3.88 (s, 3H), 3.19 (q, *J* = 8.8 Hz, 1H), 2.85 – 2.68 (m, 2H), 2.46 (s, 3H), 2.29 – 2.11 (m, 2H), 1.84 (t, *J* = 14.7 Hz, 3H), 1.66 (dd, *J* = 14.9, 8.0 Hz, 1H), 1.40 – 1.15 (m, 7H), 0.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.60, 143.51,

143.22, 138.21, 131.51, 129.63, 129.37, 127.14, 114.58, 108.04, 77.35, 77.03, 76.72, 63.35, 56.08, 51.13, 44.11, 42.92, 38.83, 36.37, 29.52, 28.90, 27.31, 26.37, 23.15, 21.57, 11.88.TOF MS ES(+)(m/z): calculated for $C_{26}H_{34}NO_4S([M+H]^+)$ 456.2209; found 456.2269.

*2.1.5.14. 2-methoxy-3-hydroxy-17β-niacinamide-1,3,5(10)-triene (**6n**)*

6n, given as a white solid; Yield: 86%; mp 152-154 ^oC; ^1H NMR (400 MHz, DMSO) δ 9.01 (d, *J* = 1.9 Hz, 1H), 8.70 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.61 (s, 1H), 8.32 – 8.09 (m, 2H), 7.59 – 7.41 (m, 1H), 6.79 (d, *J* = 21.9 Hz, 1H), 6.46 (s, 1H), 4.17 – 3.95 (m, 1H), 3.76 – 3.67 (m, 3H), 2.72 – 2.59 (m, 2H), 2.23 (dd, *J* = 26.4, 23.6 Hz, 2H), 1.94 (dd, *J* = 11.4, 6.8 Hz, 1H), 1.83 – 1.67 (m, 4H), 1.42 – 1.21 (m, 6H), 0.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.51, 152.04, 148.93, 146.02, 144.82, 135.58, 130.97, 130.82, 128.79, 123.79, 116.08, 110.28, 59.53, 56.30, 51.74, 44.39, 44.17, 37.74, 28.92, 27.63, 27.03, 26.63, 23.50, 12.82.

2.2. Biological methods

2.2.1. Cell lines culture

SKN-SH cells, SGC-7901 cells, MCF-7 cells, PC-3 cells and EC-9706 cells were obtained from School of Pharmaceutical Sciences of Zhengzhou University. SKN-SH cells were maintained in Dulbecco's Modified Eagle Medium (DMEM); SGC-7901 cells, MCF-7 cells, PC-3 cells and EC-9706 cells were kept in Roswell Park Memorial Institute 1640 (RPMI 1640). All the cells were maintained at 37 ^oC in 5 % CO_2 humidified atmosphere and supplemented with 10 % fetal bovine serum, penicillin and streptomycin.

2.2.2. Antiproliferative activity assay

The cancer cells in the exponential growth period were treated with trypsin, and diluted to 5×10^4 cells/mL. Then 100 μL of the cell suspension was transferred into 96

well microtiter plates. After cultured cells for 24 hours, certain concentration of derivatives of **5a** - **5n** and **6a** - **6n** was added in comparison to the control group. After treatment for 72 h, the cells were performed in SRB staining experiments to stain the cancer cells. The absorbance of each hole in the 96 well microtiter plates was measured by Multiskan Spectrum. Then the growth inhibition ratio was calculated according to the absorbance of each hole (the growth inhibition ratio= (1 - Absorbance of drug group/Absorbance of control group) × 100 %). The half inhibition concentration (IC_{50}) was calculated by SPSS 15. All experiments were performed six times.

2.2.3. In Vivo Pharmacokinetics Studies

Sprague–Dawley rats (200 ± 20 g) were purchased from Experimental Animal Center of Henan province, and were randomly divided into three groups (6 per group). The rats used for this study were housed individually under normal conditions, and fasted overnight before experiment with free access to water. Drugs were dissolved in a solution consisting of Cremophor EL-Anhydrous ethanol-normal saline (1:2:7). A dose of 10 mg/kg of 2-ME, **5e** and **6c** were injected through tail vein. After the injection of 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 480, 720 min, 0.5 mL of blood samples was taken from the orbital venous plexus into micro-tubes containing sodium heparin, and centrifugated immediately at 4000 rpm for 10 min, and the upper plasma was taken. Anhydrous sodium sulfate (0.1g) was added to 200 μL plasma to precipitate protein, and sodium fluoride (0.1g) was also added to the plasma collected from 2-ME derivatives group to inhibit the activity of esterase. Plasma (200 μL) was extracted with ethyl acetate (1.4 mL) vortexing for 2 min. Following centrifugation at 3000 rpm for 10 min, the organic phase was decanted to a clean tube and evaporated to dryness under a stream of air at room temperature. The dried sample was then

dissolved in 100 μL mobile phase and 20 μL of the solution was injected into the column. HPLC analysis was performed using a Phenomenex C_{18} column (5 μm, 250 × 4.6 mm) eluted with methanol-water at flow rate of 1.0 mL/min. The excited wavelength and emission wavelength were 288 and 325 nm, respectively. Column temperature is 30 ^{o}C, and the proportion of methanol-water mobile phase is 75:25 (**2-ME**), 90:10 (**5e**), 80:20 (**6c**), respectively. Pharmacokinetics parameters were evaluated using practical pharmacokinetics program version 3P97 (supplied by Chinese Pharmacological Society).

3. Results and discussion

3.1. Chemistry

There are many methods reported for the synthesis of 17-amine. Jourdan et al [16] and Gonschior et al [29] depicted a way of reduction of the oxime with $MoO_3/NaBH_4$ gave the β-amine. Cushman et al [30] and Shah [25] described that the reaction of 2-methoxy-3-benzylestrone with benzylamine afforded an intermediate Schiff base which was reduced to the secondary amine with sodium cyanoborohydride and followed by hydrogenolysis with palladium hydroxide on charcoal as the catalyst to afford the corresponding amine. Taylor et al [31] used sodium triacetoxyborohydride (STAB-H, $(CH_3COO)_3BHNa$) as reductive amination agent, then hydrogenolysis by 15 wt% of 10% Pd/C to prepare 17β-amino in high yield.

Base on above methods, the synthesis of target compounds **6a - 6n** is described in **Scheme 1**. In order to synthesize the 17β-acylamide derivatives, 2-methoxy-3-*O*-benzylestradiol **1** was prepared from estradiol following previously published methods [9].

Scheme 1 here.

First, compound **1** was oxidized to ketone **2** by Oppenauer oxidation in a yield of 82%. Then the reaction of ketone **2** with hydroxylamine in pyridine at 60 ^{o}C gave oxime **3** in a yield of 94%. Reduction amination of the oxime **3** with sodium borohydride and molybdenum trioxide (MoO_3/$NaBH_4$) in 4:1 mixture of absolute methanol and absolute tetrahydrofuran (MeOH/THF) gave 17β-amine **4** in a yield of 76%. Finally, acyl chlorides were hooked-up at 17β-amine of **4** using triethylamine in dichloromethane to afford 2-methoxy-3-benzylestra-1,3,5(10)-triene-17β-acylamide compounds **5a - 5n**, followed by debenzylation by Pd-C/H_2 in dry ethanol yielded the corresponding phenol **6a - 6n.**

The configuration of the C-17 substituent was on the basis of ^{1}H NMR spectra. The 17α and 17β protons not only exhibited different chemical shifts, but showed different coupling patterns with the amide N-H and the C-16 protons characteristic of the C-17 isomers. C-17 acetamide isomers showed the 17α-H (quasi-axial) as a quartet (d,d,d), whereas the 17β-H (quasi-equatorial) was a triplet (d,d) [32] . In the spectrum, compounds **5c** and **6c** (**Fig. 3**, **b**, **c**) gave a quartet (d,d,d) centered at 4.04 ppm corresponding to the 17a-H and the C-18 methyl signal at 0.74 ppm which were assigned to the 17α-H and the C-18 methyl in 3-acetoxy-17β-acetylamine of Lemini et al reported [32].

The triplet at 3.14 ppm (**Fig. 3a**) and the singlet at 0.96 ppm in the spectrum of compound **4** indicating that the 17-amino was obtained as its β-epimer according on Bruker Avance 400 spectrometer in $CDCl_3$, which consistent with Szendi et al reported [33] that the 17α-H (quasi-axial) was found at a higher field as a triplet signal, whereas the one corresponding to 17β-H (quasi-equatorial) appeared as a doublet.

The configuration of **6c** was also confirmed by the single crystal X-ray diffraction

(**Fig. 4**). The single crystal was crystallized from a mixture of acetone and water (ratios of the volume was 2:1) by the slow evaporation method in NMR tube at room temperature. It can be seen directly the configuration of 17-NH_2 of **6c** is β-epimer.

Fig.3. Here.

Fig. 4. Here.

3.2 Antiproliferative assay

Antiproliferative activity was assessed against six tumor cell lines (SKN-SH, SGC-7901, MCF-7, PC-3 and EC-9706). As listed in Table 1, only three compounds 3-benzyloxy-17-Chloroacetamide (**5e**), 3-benzyloxy-17-niacinamide (**5n**), and 17-butyramide (**6c**) had similar or even better effects than 2-ME in cancer cells with a broad-spectrum inhibitory effect. Compound **5e** showed better activities with the IC_{50} values of 2.27 ± 0.36 μmol/L and 2.85 ± 0.455 μmol/L, respectively against PC-3 and SK-N-SH than 2-ME. **5n** showed better activities with the IC_{50} values of 3.73 ± 0.57 μmol/L and 3.71 ± 0.57 μmol/L, respectively against SK-N-SH and EC-9706. **6c** showed better activities with an IC_{50} values of 2.95 ± 0.47 μmol/L against EC-9706 and slightly weaker activity against MCF-7 and SGC-7901 than 2-ME.

As can be seen from the data presented in Table 1, there is a general declining trend of antiproliferative activity for the 17-substituted acylamide analogs as steric bulk increases relative to 2-ME. It is consistent with the views that H-bond donating group leads to a marked decrease in antiproliferative activity, which is similar to 17-amine and 17-carboxamide 2-ME derivatives [25]. But 17-butyramide (**6c**) displayed excellent antiproliferative activities against EC-9706, being slightly less potent than 2-ME against MCF-7 and SGC-7901. However, 17-propionamide (**6b**), 17-acetamide

(**6a**), 17-pivaloylamide (**6d**) and 17-halogenated amide (**6e**, **6f**, **6g**) had little effect on antitumor activity. There may be a critical size restriction on the 17-acylamide substituent in the 2-ME derivatives that modulate the interaction of these substances with tubulin [27].

In addition, aromatic compounds (**6h**, **6j**, **6n**), halogenated aromatic compounds (**6i**, **6k**) and sulfonamide compounds (**6l**, **6m**) had poor activity, which indicated that π - electron effect at C-17 position did not help to enhance anticancer activity. Whereas 17-phenylacetamide (**6j**) showed moderate antiproliferative activities against EC-9706 and SGC-7901, 17-p-toluenesulfonamide (**6m**), 17-niacinamide (**6n**) displayed similar or better antiproliferative activities against SK-N-SH. But 17-methanesulfonamide (**6l**) was equipotent with 2-ME in EC-9706, which was very different with the Potter reported no activity in DU-145 [16].

To our surprise, when the C-3 position was benzyloxy, 17-chloroacetamide compound (**5e**) exhibited better or equivalent activity with 2-ME. Deprotected 17-chloroacetamide (**6e**) was inactive at all. These differences in activity indicate that in addition to steric effects, electronic effects are also important. As an electron donor, benzyloxy at C-3 position may change the electronic distribution of the estrogenic steroids skeleton and affect the binding ability between the colchicine binding sites of tubulin with the 2-ME analogs. This can be seen from the comparative data showed in **Table 1**. The antiproliferative activities between C-3 protection and deprotection 17-acylamide substituent compounds were just the opposite. Such as compounds **5d**, **5e**, **5f**, and **5g** exhibited similar or slightly less potent activities against cancer cell proliferation comparing with 2-ME, whereas **6d**, **6e**, **6f**, and **6g** were inactive at all. The opposite effects between **5l** and **6l**, **5m** and **6m** can also be seen from the table 1. In addition, **5n** and **6n**, **5c** and **6c** displayed comparable antiproliferative activities.

Conversely, **5a** and **6a**, **5b** and **6b** were inactive at all.

From the above SAR studies, we found that single electronic effects, steric effects, or H-bond donor at C-3 or C-17 positions were not sufficient to heighten antitumor activity with acceptable metabolic stability. Perhaps only with suitable steric effects, electronic effects, H-bond donor at C-17 position and H-bond acceptor at C-3 position can help enhance metabolic stability, and increase or maintain the anticancer activity.

Table 1 here.

3.3. In Vivo Pharmacokinetics Studies

Based on their antiproliferative activities profiles in vitro, **5e** and **6c** displayed considerably similar or improved antiproliferative activity compared with 2-ME, so we chose compounds **5e** and **6c** for metabolic stability studies. Rat intravenous injection was used as an initial screen for PK evaluation in the experiment (**Fig. 5**). The elimination half-life ($t_{1/2}$), plasma concentration-time curve (AUC), clearance and volume of distribution of the two analogs was calculated via compartmental analyses using 3P97 Software (**Table 2**).

Fig. 5 Here.

Table 2 here.

In vivo pharmacokinetics studies data indicated that 2-ME, **5e**, and **6c** followed a two-compartment model with different pharmacokinetics parameters after i.v. administration of at a dose of 10 mg/kg to rats (**Table 2**). **Fig. 5** indicated that

comparation in the curve of plasma concentration to time after injecting 2-ME, **5e**, and **6c**. The comparison of other parameters within plasma showed that 2-ME had a lower distribution half-life $t_{1/2\alpha}$ (2.70 ± 1.31 min) and elimination half-life $t_{1/2\beta}$ (22.28 ± 2.59 min) than the **5e**, and **6c**. It can be seen directly that **5e** had a similar plasma concentration-time curve compared with 2-ME with elimination half-life $t_{1/2\beta}$ (29.50 ± 4.46 min) from **Fig. 5**. In other words, like its progenitor compound 2-ME, **5e** was rapidly metabolized *in vivo*, whereas **6c** was still detectable in the plasma after 12 h of exposure. In **Table 2**, pharmacokinetics analysis of rats intravenously injected with **6c** demonstrated a $t_{1/2\alpha}$ of 40.19 min, a $t_{1/2\beta}$ of 240.93 min, the $AUC_{0\text{-}tmin}$ (2068.20 ± 315.74) $\mu g \cdot mL^{-1} \cdot min$, a clearance of 0.0047 ml/min. The elimination half-life of **6c** was ten times longer than 2-ME and area under the curve was seven times higher than 2-ME, respectively, which meant that **6c** had higher bioavailability and longer metabolism than 2-ME. Taken together, compound **6c** had superior bioavailability and might be useful for further *in vivo* studies.

4. Conclusion

Twenty-eight derivatives of **5a-5n** and **6a-6n** were synthesized, and cell activity tests were carried out. Three compounds **5e**, **5n** and **6c** were found to have similar or better anticancer activity with 2-ME. But *in vivo* pharmacokinetics study showed compound **6c** had superior bioavailability and prolonged metabolism against S180 mouse ascites tumor. The elimination half-life of 6c ($t_{1/2\beta}$ = 240.93 min) is ten times longer than 2-ME ($t_{1/2\beta}$ = 22.28 min) and the area under the curve was seven times ($AUC_{0\text{-}tmin}$ = 2068.20 ± 315.74 $\mu g \cdot ml^{-1} \cdot min$) higher than 2-ME ($AUC_{0\text{-}tmin}$ = 263.57 ± 93.83 $\mu g \cdot ml^{-1} \cdot min$), respectively, which meant C17-amide is helpful in prolonging metabolism and in improving bioavailability and C17-amide in place of C17-OH afforded us a useful direction to synthesize 2-ME derivatives.

Taking into consideration the suitable steric effects, electronic effects, hydrogen bond donor, and hydrogen bond acceptor, we can provide and design new types of compounds to improve the antiproliferative activity.

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Tables

Table 1. The IC_{50} of some derivatives of **5a-5n** and **6a-6n** against five cancer cells.

Compound	PC-3	SK-N-SH	EC-9706	MCF-7	SGC-7901
			IC_{50} (μmol/L)		
2-ME	6.79 ± 0.83	8.69 ± 0.94	4.13 ± 0.62	5.77 ± 0.76	3.52 ± 0.26
5a	>50	>50	>50	>50	>50
5b	28.75 ± 1.46	28.86 ± 1.46	29.19 ± 1.46	>50	20.82 ± 1.32
5c	18.97 ± 1.28	13.58 ± 1.13	9.82 ± 0.99	17.4 ± 9.95	12.95 ± 1.11
5d	>50	6.12 ± 0.79	13.46 ± 1.13	33.06 ± 1.52	19.73 ± 1.30
5e	2.27 ± 0.36	2.85 ± 0.455	4.65 ± 1.56	15.7 ± 1.20	7.87 ± 0.98
5f	>50	13.90 ± 1.14	21.70 ± 1.34	11.32 ± 1.05	4.70 ± 0.67
5g	>50	6.59 ± 0.82	20.93 ± 1.32	16.34 ± 1.32	15.63 ± 1.19
5h	>50	35.15 ± 1.55	>50	30.68 ± 2.64	>50
5i	48.21 ± 1.68	>50	26.49 ± 3.81	>50	>50
5j	27.79 ± 1.44	6.20 ± 0.79	>50	>50	46.67 ± 1.67
5k	38.21 ± 1.72	9.09 ± 0.96	>50	>50	25.38 ± 1.40
5l	>50	10.84 ± 1.04	>50	45.0 ± 1.81	>50
5m	>50	>50	43.44 ± 1.64	32.32 ± 2.09	>50
5n	>50	3.73 ± 0.57	3.71 ± 0.57	9.86 ± 0.99	7.64 ± 0.88
6a	>50	>50	36.02 ± 2.42	>50	>50
6b	>50	39.94 ± 1.60	>50	45.5 ± 6.48	>50
6c	10.38 ± 1.02	20.87 ± 1.32	2.95 ± 0.47	8.03 ± 0.90	6.03 ± 0.78
6d	>50	30.68 ± 1.49	>50	>50	>50
6e	>50	>50	>50	>50	>50
6f	>50	>50	30.63 ± 1.49	49.9 ± 3.48	>50
6g	>50	>50	>50	>50	>50
6h	>50	22.13 ± 0.46	16.90 ± 1.84	22.91 ± 1.63	9.30 ± 0.98
6i	>50	45.5 ± 5.48	>50	29.05 ± 2.40	33.2 ± 4.35
6j	36.27 ± 1.56	36.56 ± 1.56	9.50 ± 0.98	>50	7.23 ± 0.86
6k	>50	35.03 ± 1.54	>50	>50	25.38 ± 1.40
6l	31.95 ± 1.50	15.13 ± 1.18	4.61 ± 0.66	>50	10.09 ± 1.00
6m	>50	8.37 ± 0.92	>50	15.67 ± 1.20	>50
6n	27.2 ± 2.06	6.59 ± 0.82	16.12 ± 1.21	11.5 ± 2.52	12.81 ± 1.11

Table 2. The pharmacokinetic parameters of **5e**, **6c** and 2-ME after intravenous administration at 10 mg/kg to mice (n = 6).

Parameters	Unit	**2-ME**	**5e**	**6c**
α	min^{-1}	0.25 ± 0.09	0.22 ± 0.01	0.0212 ± 0.01
β	min^{-1}	0.03 ± 0.0036	0.02 ± 0.01	0.0029 ± 0.00
$t_{1/2\alpha}$	min	2.70 ± 1.31	3.84 ± 0.21	40.19 ± 19.06
$t_{1/2\beta}$	min	22.28 ± 2.59	29.50 ± 4.46	240.93 ± 25.64
Vc	$(mg{\cdot}kg^{-1})/(\mu g{\cdot}ml^{-1})^{-1}$	0.55 ± 0.41	0.38 ± 0.14	0.74 ± 0.20
CL(s)	$mg{\cdot}kg^{-1}{\cdot}min^{-1}{\cdot}(\mu g{\cdot}ml^{-1})^{-1}$	0.04 ± 0.02	0.03 ± 0.01	0.0047 ± 0.001
$AUC_{0\text{-}tmin}$	$\mu g{\cdot}ml^{-1}{\cdot}min$	263.57 ± 93.83	251.06 ± 64.54	2068.20 ± 315.74
$MRT_{0\text{-}tmin}$	min	24.68 ± 2.00	29.77 ± 2.53	181.61 ± 11.18

Figure legends

Fig. 1. Molecular structures of 2-ME, STX140 and ENMD-1198.

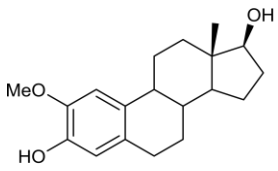
Fig. 2. Some amino analogues of 2-ME modification on C-17 in ring D.

Scheme 1. Reagents and conditions: (i) Aluminum isopropoxide, cyclohexanone, toluene, reflux, 24 h; (ii) $NH_2OH{\cdot}H_2O$, pyridine, 60°C, 1 h; (iii) $NaBH_4$, MoO_3, MeOH, THF, 0 °C, 24 h; (iv) Acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 1 h; (v) Pd/C, H_2, EtOH, 60 °C, 4 h.

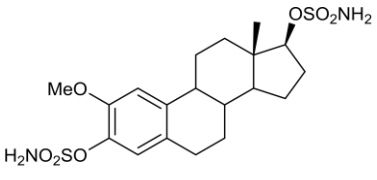
Fig. 3. 400 MHz ^1H NMR of 17-H of compound in $CDCl_3$: (a) Epimer 17β-amino of **4**; (b) Epimer 17β-butyrylamino of **5c**; (c) Epimer 17β-butyrylamino of **6c**.

Fig. 4. ORTEP-type plot of the 6c molecule. Ellipsoid are drawn at 30% probability level. The single crystal was crystallized from a mixture of acetone and water (ratios of the volume was 2:1).

Fig. 5. The plasma concentration-time curve of **5e**, **6c** and 2-ME (n=6).



2-ME



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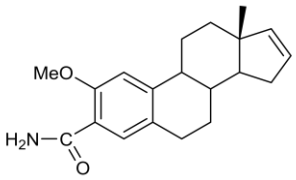

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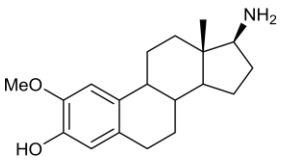

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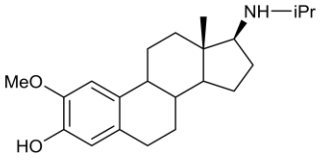

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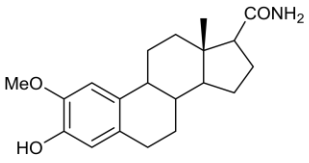

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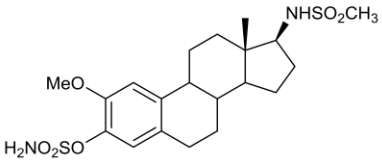

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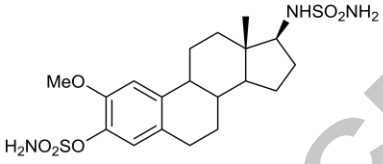

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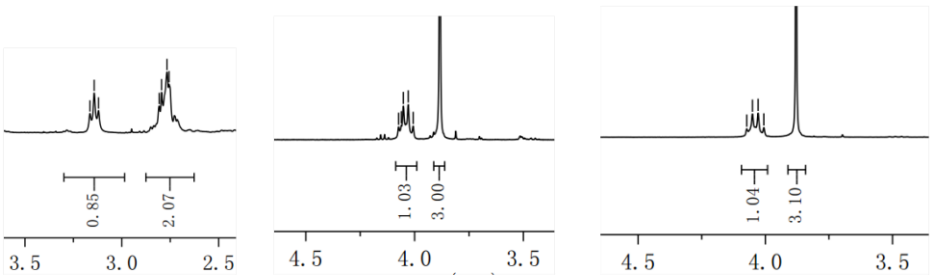
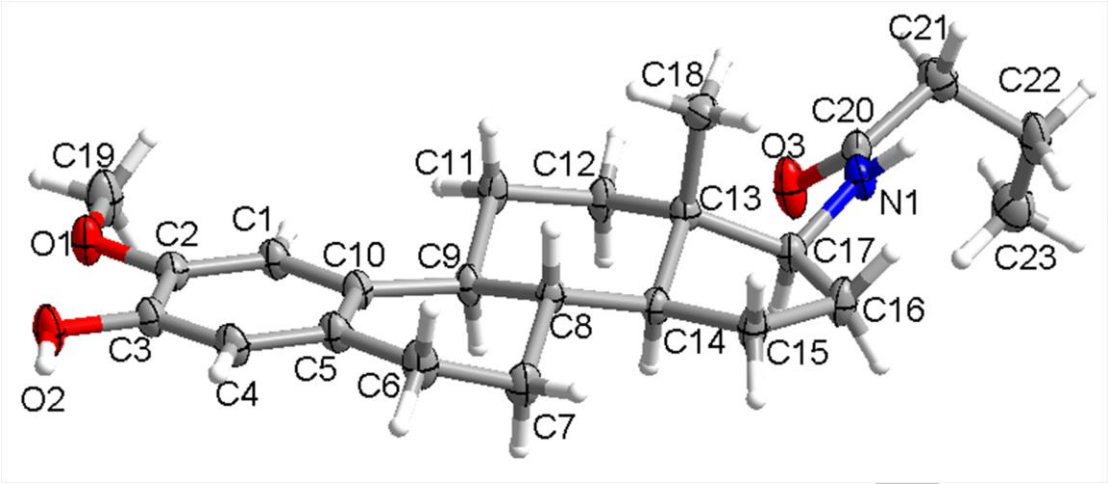
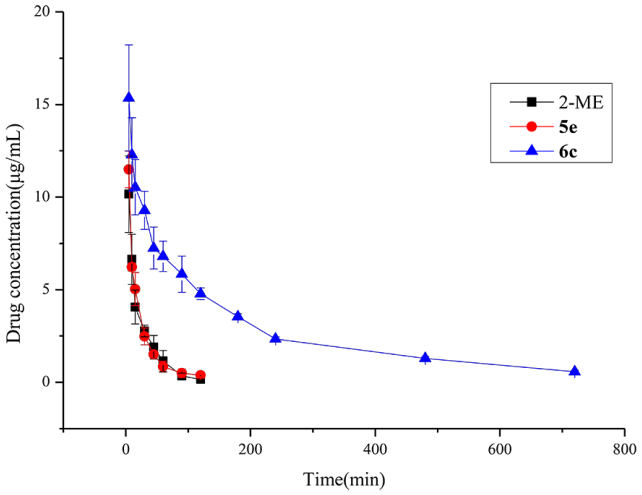
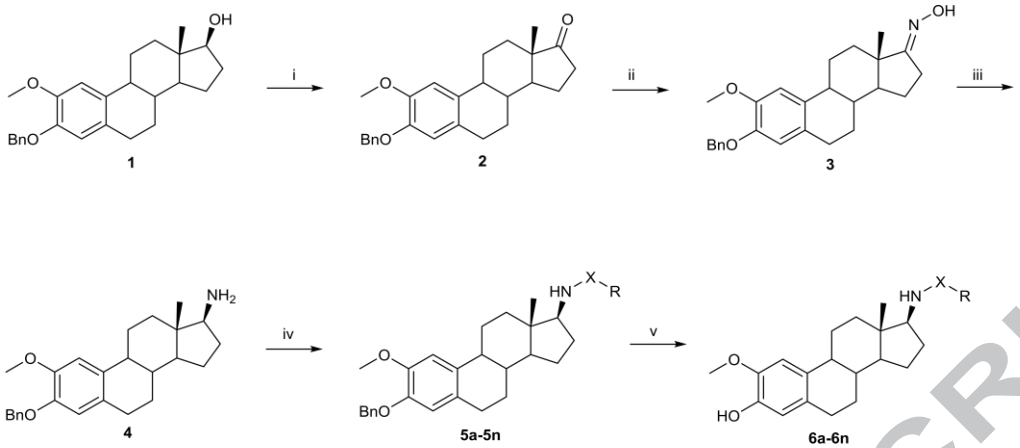


(a) **(b)** **(c)**

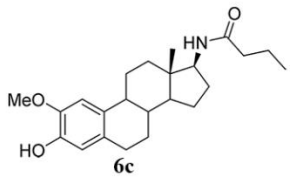




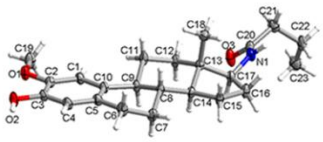


5a: X=CO, R=CH_3
5b: X=CO, R=CH_2CH_3
5c: X=CO, R=$CH_2CH_2CH_3$
5d: X=CO, R=$C(CH_3)_3$
5e: X=CO, R=CH_2Cl
5f : X=CO, R=CH_2CH_2Cl
5g: X=CO, R=CH_2CH_2F
5h : X=CO, R=Ph
5i : X=CO, R=4-F-Ph
5j : X=CO, R=CH_2Ph
5k : X=CO, R=2,3,4,5-tetrafluoro-Ph
5l : X=SO_2, R=CH_3
5m: X=SO_2, R=4-CH_3-Ph
5n : X=CO, R=3-pyridine

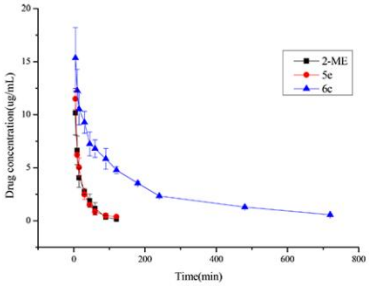
6a: X=CO, R=CH_3
6b: X=CO, R=CH_2CH_3
6c: X=CO, R=$CH_2CH_2CH_3$
6d: X=CO, R=$C(CH_3)_3$
6e: X=CO, R=CH_2Cl
6f : X=CO, R=CH_2CH_2Cl
6g: X=CO, R=CH_2CH_2F
6h : X=CO, R=Ph
6i : X=CO, R=4-F-Ph
6j : X=CO, R=CH_2Ph
6k : X=CO, R=2,3,4,5-tetrafluoro-Ph
6l : X=SO_2, R=CH_3
6m: X=SO_2, R=4-CH_3-Ph
6n : X=CO, R=3-pyridine



6c



The single crystal of **6c**



The plasma concentration-time curve of **5e**, **6c** and 2-ME

First reported 17β-amide derivatives of 2-methoxyestradiol.

The single crystal X-ray diffraction confirmed the configuration of 17β-butyrylamino of 6c.

Three derivatives had similar or even better effects than 2-ME in antiproliferative assay.

Pharmacokinetic tests showed that the half-life of **6c** was ten times that of 2ME.