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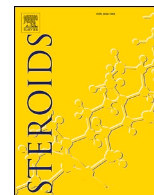

# Efficient access to domain-integrated estradiol-flavone hybrids via the corresponding chalcones and their *in vitro* anticancer potential

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Dedicated to Professor György Keglevich on his 65th birthday.

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ABSTRACT

Structural modification of the phenolic A-ring of estrogens at C-2 and/or C-3 significantly reduces or eliminates the hormonal effects of the compounds, thus the incorporation of other pharmacophores into these positions can provide biologically active derivatives suitable for new indications, without possessing unwanted side effects. As part of this work, A-ring integration of estradiol with chalcones and flavones was carried out in the hope of obtaining novel molecular hybrids with anticancer action. The syntheses were performed from 2-acetylestradiol-17β-acetate which was first reacted with various (hetero)aromatic aldehydes in a pyrrolidine-catalyzed reaction in DMSO. The chalcones thus obtained were then subjected to oxidative cyclization with $I_2$ in DMSO to afford estradiol-flavone hybrids in good yields. All newly synthesized derivatives were tested *in vitro* for cytotoxicity on human malignant cell lines of diverse origins as well as on a non-cancerous cell line, and the results demonstrated that estradiol-flavone hybrids containing a structure-integrated flavone moiety were the most active and cancer cell-selective agents. The minimal inhibitory concentration values ($IC_{50}$) were calculated for selected compounds (**3c**, **3d** and **3e**) and their apoptosis inducing capacity was verified by RT-qPCR (real-time quantitative polymerase chain reaction). The results suggest an important structure–activity relationship regarding estradiol-flavone hybrids that could form a promising synthetic platform and rationale for future drug developments.

## 1. Introduction

The concept of molecular hybridization, which involves the creation of a new molecule by combining two or more structural subunits of different bioactive compounds, offers an excellent opportunity for the chemical modifications of natural compounds [1–7]. Hybrid drugs provide a state-of-the-art therapeutic option for a variety of diseases, such as cancer, and unlike many conventional treatments, may display higher and more selective activity, including the elimination of drug resistance and the reduction of unwanted side effects. A number of molecular hybrids, also known as "chimeras", consisting of natural steroids and other pharmacophores through the domain integration of key functional elements or linked by a covalent bond, have been reported so far [8–14].

Among the hybridization strategies, the introduction of heterocyclic systems, whether attached or fused to the sterane backbone, can significantly modify the biological and pharmacokinetic behavior of the parent compound [15,16]. The incorporation of heterocycles generally improves water solubility, intestinal absorption, and stability against metabolic degradation. In addition to the properties listed, it should not be ignored that hybrid compounds derived from sex steroids may still be able to bind to estrogen or androgen receptors, thereby causing undesirable hormonal effects. Accordingly, semi-synthetic structural modifications affecting the A- and D-rings of the sterane skeleton responsible for hormonal activity are most reasonable.

As previously demonstrated, the substitution of the aromatic A-ring of estrogens on C-2 with larger groups, as well as those that might involve the 3-OH group in an intramolecular H-bond, substantially reduces the estrogen receptor binding, and thus the hormonal effect of such molecules [17]. Decreased or disrupted receptor binding can be attributed to a number of factors, including distortion of the sterane skeleton, alterations in the phenolic $pK_a$, changes in the electronic

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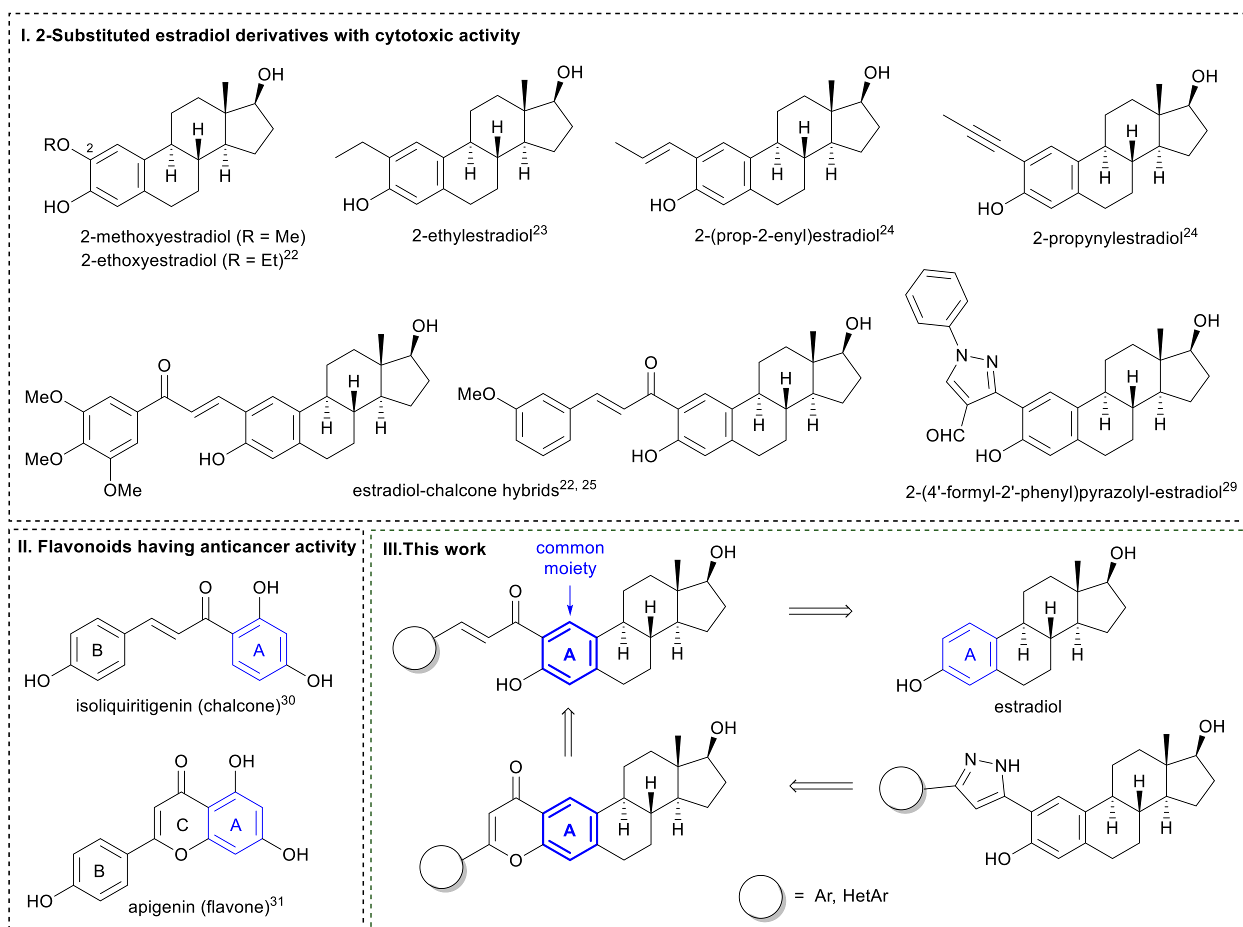



**Fig. 1.** A-ring substituted and hybridized derivatives of estradiol.

properties of the aromatic moiety, and steric hindrance caused by the introduced substituent. Since the 3-OH of estrogens interacts with the hormone receptor primarily as a H-bond donor, analogs that have functionalities at position 3 acting as H-acceptors also have low affinity to the target protein.

Several A-ring-substituted estradiol derivatives, the most studied of which is 2-methoxyestradiol (Fig. 1/I.), were found to have anticancer activity with reduced or eliminated hormonal effect [18–21]. This molecule inhibits cellular mechanisms involved in the stabilization of microtubules, and functions also as an antiangiogenic agent, *i.e.*, prevents the growth of new blood vessels in cancer cells. However, its unfavorable pharmacokinetic profile (low half-life, *in vivo* metabolism to estradiol, rapid elimination) has motivated the synthesis and pharmacological investigation of various analogs in recent decades [18,21]. Replacement of the OMe group resulted in a number of 2-substituted compounds, including chalcone hybrids, some of which showed marked anticancer activity (Fig. 1/I.) [22–25]. Interestingly, only few examples are to be found in the literature for the preparation and pharmacological study of 2-connected [26] and 2,3-fused heterocyclic estradiol derivatives [27,28]. In this regard, we have recently reported a high-yield synthetic procedure for the preparation of 2-pyrazolyl estradiols and estradiol-pyrazolocoumarin hybrids [29].

The aim of the present study was to develop an efficient route for the synthesis of A-ring-integrated estradiol-flavone hybrids through the corresponding chalcones and their subsequent transformation to 2-pyrazolyl derivatives by ring opening reaction. This multistep sequence resulted in three families of estradiol hybrid compounds (Fig. 1/III.) that may be worthy of anticancer studies for the following reasons in addition to the above. A great number of naturally occurring flavonoids, including chalcones (*e.g.* isoliquiritigenin, xanthohumol, licochalcone A) and flavones (*e.g.* apigenin, chrysin, tangeretin) as well as their synthetic analogs – alone or as part of a hybrid compound – are known to exhibit cytotoxic activity against a variety of cancer cell lines in low micromolar range (Fig. 1/II.) [30–32]. In addition, the incorporation of a pyrazole moiety into position 2 of the estrane framework also led to some effective anticancer agents (Fig. 1/I.) [29]. Consequently, the cytotoxic activity of the synthesized compounds were tested *in vitro* on cancerous MCF-7, HeLa, DU-145, PC-3 and on non-cancerous MRC-5 cell lines. Furthermore, based on the toxicity data, the most promising candidates were selected and tested for their apoptosis-inducing potential by real-time qPCR.

## 2. Materials and methods

### 2.1. Synthesis of the prepared compounds

#### 2.1.1. General

Chemicals, reagents, and solvents were purchased from commercial suppliers (Sigma-Aldrich and Alfa Aesar) and used without further purification. Reactions under MW irradiation were carried out with a CEM Corporation Focused Microwave System, Model Discover SP. Melting points (Mp) were determined on an SRS Optimelt digital apparatus and are uncorrected. The transformations were monitored by TLC using 0.25 mm thick Kieselgel-G plates (Si 254 F, Merck). Compound spots were detected by spraying with 5 % phosphomolybdic acid in 50 % aqueous phosphoric acid. Flash chromatographic purifications were carried out on silica gel 60, 40–63 µm (Merck). All eluent and solvent system compositions are given in volume percent (v/v%). NMR spectra were recorded with a Bruker DRX 500 instrument at room temperature in $CDCl_3$ or DMSO-$d_6$ using residual solvent signals as an internal

reference. Chemical shifts are reported in ppm (δ scale) and coupling constants (*J*) are given in Hz. Multiplicities of the $^{1}$H signals are indicated as a singlet (s), a doublet (d), doublet of doublets (dd), a triplet (t) or a multiplet (m). $^{13}$C NMR spectra are $^{1}$H-decoupled and the J-MOD pulse sequence was used for multiplicity editing. In this spin-echo type experiment, the signal intensity is modulated by the different coupling constants *J* of carbons depending on the number of attached protons. Both protonated and unprotonated carbons can be detected ($CH_3$ and CH carbons appear as positive signals, while $CH_2$ and C carbons as negative signals). Elemental analysis data were obtained with a Perkin Elmer CHN analyzer model 2400. The purified derivatives were dissolved in high purity acetonitrile and introduced with an Agilent 1290 Infinity II liquid chromatography pump to an Agilent 6470 tandem mass spectrometer equipped an electrospray ionization chamber. Flow rate was 0.5 mL·min$^{-1}$, and contained 0.1 % formic acid or 0.1 % ammonium hydroxide to help facilitate ionization. The instrument operated in MS1 scan mode with 135 V fragmentor voltage, and the spectra were recorded from 200 to 600 *m/z*, which were corrected with the background.

*2.1.2. General procedure for the synthesis of chalcones (**2a-k**)*

To a solution of 2-acetyl-estradiol-17β-acetate (**1**, 356 mg, 1.0 mmol) in DMSO (10 mL), the corresponding aldehyde (1.5 mmol) and pyrrolidine (2 drops) were added. The resulting deep red solution was stirred at 60 °C for 3 h or until complete conversion (TLC monitoring). The reaction mixture was then poured into cold water and the resulting yellow precipitate was filtered off and dried. The crude product was redissolved in methanolic KOH (1 M, 10 mL) solution and was stirred for 1 h at room temperature. The reaction mixture was then poured into cold water again, made slightly acidic with 10 % HCl and extracted with EtOAc (2 × 25 mL). The combined organic phases were washed with brine, dried over anhydrous $Na_2SO_4$ and reduced in vacuo. The resulting yellow-orange solid was purified by column chromatography.

*2.1.2.1. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-furyl-prop-2′-en-1′-one (**2a**).* According to the general procedure, 2-furaldehyde (0.12 mL) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 2:98 to afford **2a** (290 mg, 74 %) as a yellow solid. Mp > 100 °C (decomposes); anal. calcd. for $C_{25}H_{28}O_4$ C, 76.50; H, 7.19; found C, 76.59; H, 7.21; $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.17–1.65 (overlapping m, 7H), 1.67–1.77 (m, 1H), 1.85–1.94 (m, 1H), 1.98–2.08 (m, 1H), 2.07–2.24 (m, 2H), 2.38–2.46 (m, 1H), 2.81–2.95 (m, 2H, 6-$H_2$), 3.76 (t, *J* = 8.5 Hz, 1H, 17-H), 6.54 (dd, *J* = 1.8, 3.4 Hz, 1H, 4″-H), 6.69–6.78 (m, 2H, 4-H and 3″-H), 7.52 (d, *J* = 15.2 Hz, 1H, 2′-H), 7.58 (d, *J* = 1.8 Hz, 1H, 5″-H), 7.66 (d, *J* = 15.1 Hz, 1H, 3′-H), 7.77 (s, 1H, 1-H), 12.68 (bs, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.5 ($CH_2$), 27.0 ($CH_2$), 30.1 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.8 (CH), 43.3 (C-13), 43.8 (CH), 50.2 (CH), 82.0 (C-17), 113.0 (C-4″), 117.0 (C-3″), 117.87 (C-4), 117.93 (C-3′), 118.3 (C-2), 126.3 (C-1), 130.8 (C-2′), 131.6 (C-10), 145.4 (C-5″), 147.4 (C-5), 151.8 (C-2″), 161.3 (C-3), 193.0 (C-1′); ESI-MS 393.2 [M + H]$^{+}$.

*2.1.2.2. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-thiophenyl-prop-2′-en-1′-one (**2b**).* According to the general procedure, 2-thiophenecarboxaldehyde (0.14 mL) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 2:98 to afford **2b** (282 mg, 69 %) as a yellow solid. Mp 193–195 °C; anal. calcd. for $C_{25}H_{28}O_3S$ C, 73.50; H, 6.91; found C, 73.38; H, 6.85; $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.16–1.28 (m, 1H), 1.26–1.66 (overlapping m, 6H), 1.67–1.77 (m, 1H), 1.86–1.94 (m, 1H), 1.99–2.08 (m, 1H), 2.08–2.25 (overlapping m, 2H), 2.35–2.43 (m, 1H), 2.81–2.95 (m, 2H, 6-$H_2$), 3.76 (t, *J* = 8.5 Hz, 1H), 6.73 (s, 1H, 4-H), 7.12 (dd, *J* = 3.6, 5.1 Hz, 1H, 4″-H), 7.37–7.45 (overlapping m, 2H, 1H of 2′-H, d, *J* = 15.1 Hz and 1H of 3″-H, d, *J* = 5.1 Hz), 7.46 (d, *J* = 5.1 Hz, 1H, 5″-H), 7.73 (s, 1H, 1-H), 8.02 (d, *J* = 15.1 Hz, 1H, 3′-H), 12.62 (bs, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.5 ($CH_2$), 27.0 ($CH_2$), 30.2 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.8 (CH), 43.3 (C-13), 43.7 (CH), 50.2 (CH), 82.0 (C-17), 117.9 (C-4), 118.2 (C-2), 119.3 (C-3″), 126.1 (C-1), 128.6 (C-2′), 129.4 (C-4″), 131.6 (C-10), 132.5 (C-5″), 137.4 (C-3′), 140.4 (C-2″), 147.5 (C-5), 161.3 (C-3), 192.9 (C-1′); ESI-MS 407.0 [M−H]$^{-}$.

*2.1.2.3. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-phenyl-prop-2′-en-1′-one (**2c**).* According to the general procedure, benzaldehyde (0.15 mL) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 2:98 to afford **2c** (315 mg, 78 %) as a yellow solid. Mp 205–207 °C; anal. calcd. for $C_{27}H_{30}O_3$ C, 80.56; H, 7.51; found C, 80.61; H, 7.42; $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.17–1.66 (overlapping m, 7H), 1.68–1.77 (m, 1H), 1.87–1.94 (m, 1H), 2.00–2.05 (m, 1H), 2.08–2.26 (m, 2H), 2.36–2.44 (m, 1H), 2.82–2.96 (m, 2H, 6-$H_2$), 3.76 (t, *J* = 8.5 Hz, 1H, 17-H), 6.75 (s, 1H, 4-H), 7.42–7.49 (m, 3H, 3″-, 4″- and 5″-H), 7.63 (d, *J* = 15.5 Hz, 1H, 2′-H), 7.65–7.70 (m, 2H, 2″- and 6″-H), 7.79 (s, 1H, 1-H), 7.90 (d, *J* = 15.5 Hz, 1H, 3′-H), 12.61 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.6 ($CH_2$), 27.0 ($CH_2$), 30.1 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.7 (CH), 43.3 (C-13), 43.7 ($CH_2$), 50.2 ($CH_2$), 82.0 (C-17), 118.0 (C-4), 118.3 (C-2), 120.5 (C-2′), 126.3 (C-4″), 128.8 (2C, C-2″ and C-6″), 129.2 (2C, C-3″ and C-5″), 130.9 (C-1), 131.6 (C-10), 134.9 (C-1″), 145.1 (C-3′), 147.5 (C-5), 161.4 (C-3), 193.5 (C-1′); ESI-MS 401.0 [M−H]$^{-}$.

*2.1.2.4. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(4″-methylphenyl)-prop-2′-en-1′-one (**2d**).* According to the general procedure, *p*-tolualdehyde (0.18 mL) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 2:98 to afford **2d** (358 mg, 86 %) as a yellow solid. Mp 199–201 °C; anal. calcd. for $C_{28}H_{32}O_3$ C, 80.73; H, 7.74; found C, 80.71; H, 7.75; $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.18–1.26 (m, 1H), 1.29–1.66 (overlapping m, 6H), 1.67–1.77 (m, 1H), 1.86–1.95 (m, 1H), 2.00–2.04 (m, 1H), 2.07–2.25 (m, 2H), 2.36–2.44 (overlapping m, 4H, 3H of 4″-$CH_3$), 2.81–2.95 (m, 2H, 6-$H_2$), 3.76 (t, *J* = 8.5 Hz, 1H, 17-H), 6.74 (s, 1H, 4-H), 7.25 (overlapping with solvent, d, *J* = 7.9 Hz, 2H, 2″- and 6″-H), 7.55–7.61 (m, 3H, 2H of 3″-, 5″-H and d, *J* = 15.4 Hz, 1H, 2′-H), 7.78 (s, 1H, 1-H), 7.88 (d, *J* = 15.4 Hz, 1H, 3′-H), 12.65 (bs, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 21.7 (4″-$CH_3$), 23.3 ($CH_2$), 26.6 ($CH_2$), 27.0 ($CH_2$), 30.1 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.8 (CH), 43.3 (C-13), 43.8 (CH), 50.2 (CH), 82.0 (C-17), 117.9 (C-4), 118.3 (C-2), 119.5 (C-2′), 126.3 (C-1), 128.8 (2C, C-3″ and C-5″), 129.9 (2C, C-2″ and C-6″), 131.6 (C-1″), 132.2 (C-10), 141.5 (C-4″), 145.1 (C-3′), 147.4 (C-5), 161.4 (C-3), 193.6 (C-1′); ESI-MS 415.0 [M−H]$^{-}$.

*2.1.2.5. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(4″-bromophenyl)-prop-2′-en-1′-one (**2e**).* According to the general procedure, 4-bromobenzaldehyde (277 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 2:98 to afford **2e** (279 mg, 58 %) as a yellow solid. Mp 201–203 °C; anal. calcd. for $C_{27}H_{29}BrO_3$ C, 67.36; H, 6.07; found C, 67.24; H, 5.99; $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.17–1.28 (m, 1H), 1.29–1.56 (overlapping m, 5H), 1.55–1.66 (m, 1H), 1.67–1.78 (m, 1H), 1.87–1.95 (m, 1H), 1.99–2.06 (m, 1H), 2.08–2.25 (overlapping m, 2H), 2.35–2.43 (m, 1H), 2.82–2.95 (m, 2H, 6-$H_2$), 3.75 (td, *J* = 5.5, 8.4 Hz, 1H, 17-H), 6.75 (s, 1H, 4-H), 7.50–7.56 (m, 2H), 7.55–7.63 (m, 3H), 7.75 (s, 1H, 1-H), 7.82 (d, *J* = 15.4 Hz, 1H, 3′-H), 12.52 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.6 ($CH_2$), 27.0 ($CH_2$), 30.2 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.8 (CH), 43.4 (C-13), 43.7 (CH), 50.2 (CH), 82.0 (C-17), 118.0 (C-4), 118.2 (C-2), 121.1 (C-2′), 125.2 (C-4″), 126.2 (C-1), 130.1 (2C, C-2″ and C-6″), 131.7 (C-10), 132.4 (2C, C-3″ and C-5″), 133.9 (C-1″), 143.6 (C-3′), 147.8 (C-5), 161.5 (C-3), 193.2 (C-1′); ESI-MS 479.0 [M−H]$^{-}$, 481.0 [M−H]$^{-}$.

*2.1.2.6. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(4″-hydroxyphenyl)-prop-2′-en-1′-one (**2f**).* According to the general procedure, 4-hydroxybenzaldehyde (183 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 20:80 to afford **2f** (285 mg, 68 %) as

a yellow solid. Mp 255–257 °C; anal. calcd. for $C_{27}H_{30}O_4$ C, 77.48; H, 7.23; found C, 77.42; H, 7.18; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.68 (s, 3H, 18-$H_3$), 1.08–1.47 (overlapping m, 7H), 1.54–1.64 (m, 1H), 1.75–1.83 (m, 1H), 1.83–1.95 (m, 2H), 2.09–2.18 (m, 1H), 2.55–2.62 (m, 1H), 2.74–2.87 (m, 2H, 6-$H_2$), 3.54 (t, $J$ = 8.5 Hz, 1H, 17-H), 4.50 (bs, 1H, 17-OH), 6.66 (s, 1H, 4-H), 6.82–6.89 (d-like m, 2H), 7.74–7.84 (m, 4H), 7.95 (s, 1H, 1-H), 10.17 (bs, 1H, 4″-OH), 12.72 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta_C$ 11.2 (C-18), 22.7 ($CH_2$), 25.8 ($CH_2$), 26.3 ($CH_2$), 29.5 ($CH_2$), 29.9 ($CH_2$), 36.6 ($CH_2$), 38.4 (CH), 42.8 (C-13), 43.4 (CH), 49.5 (CH), 80.0 (C-17), 115.8 (2C, C-3″ and C-5″), 117.0 (C-2′), 117.5 (C-4), 118.3 (C-2), 125.6 (C-1″), 126.8 (C-1), 131.48 (C-10), 131.55 (2C, C-2″ and C-6″), 145.3 (C-3′), 146.5 (C-5), 160.0 (C-4″), 160.5 (C-3), 193.2 (C-1′); ESI-MS 417.0 [M−H]$^-$.

*2.1.2.7. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(4″-methoxyphenyl)-prop-2′-en-1′-one **(2g)**.* According to the general procedure, 4-methoxybenzaldehyde (0.18 mL) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 2:98 to afford **2g** (342 mg, 79 %) as a yellow solid. Mp 205–207 °C; anal. calcd. for $C_{28}H_{32}O_4$ C, 77.75; H, 7.46; found C, 77.64; H, 7.44; $^1$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.17–1.29 (m, 1H), 1.29–1.66 (overlapping m, 6H), 1.67–1.77 (m, 1H), 1.86–1.95 (m, 1H), 1.99–2.06 (m, 1H), 2.09–2.25 (overlapping m, 2H), 2.36–2.44 (m, 1H), 2.81–2.95 (m, 2H, 6-$H_2$), 3.76 (dt, $J$ = 6.4, 12.9 Hz, 1H, 17-H), 3.87 (s, 3H, 4″-$OCH_3$), 6.74 (s, 1H, 4-H), 6.93–7.00 (d-like m, 2H, 2″- and 6″-H), 7.50 (d, $J$ = 15.4 Hz, 1H, 2′-H), 7.60–7.67 (d-like m, 2H, 3″- and 5″-H), 7.78 (s, 1H, 4-H), 7.88 (d, $J$ = 15.4 Hz, 1H, 3′-H), 12.70 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.6 ($CH_2$), 27.0 ($CH_2$), 30.1 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.8 (CH), 43.4 (C-13), 43.8 (CH), 50.2 (CH), 55.6 (4″-$OCH_3$), 82.0 (C-17), 114.6 (2C, C-2″ and C-6″), 117.9 (C-4), 118.0 (C-2′), 118.4 (C-2), 126.2 (C-1), 127.7 (C-1″), 130.6 (2C, C-3″ and C-5″), 131.5 (C-10), 144.9 (C-3′), 147.2 (C-5), 161.4 (C-3), 162.1 (C-4″), 193.5 (C-1′); ESI-MS 433.2 [M + H]$^+$.

*2.1.2.8. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(4″-hydroxy-3″-methoxyphenyl)-prop-2′-en-1′-one **(2h)**.* According to the general procedure, vanillin (228 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 20:80 to afford **2h** (269 mg, 60 %) as a yellow solid. Mp 190–192 °C; anal. calcd. for $C_{28}H_{32}O_5$ C, 74.98; H, 7.19; found C, 74.93; H, 7.20; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.68 (s, 3H, 18-$H_3$), 1.09–1.44 (overlapping m, 7H), 1.54–1.64 (m, 1H), 1.75–1.83 (m, 1H), 1.89 (dt, $J$ = 3.5, 13.6 Hz, 2H), 2.10–2.19 (m, 1H), 2.54–2.61 (m, 1H), 2.78–2.88 (m, 2H, 6-$H_2$), 3.54 (t, $J$ = 8.6 Hz, 1H, 17-H), 3.86 (s, 3H, 3″-$OCH_3$), 4.49 (s, 1H, 17-OH), 6.67 (s, 1H, 4-H), 6.86 (d, $J$ = 8.2 Hz, 1H, 5″-H), 7.42 (dd, $J$ = 2.0, 8.3 Hz, 1H, 6″-H), 7.48 (d, $J$ = 2.0 Hz, 1H, 2″-H), 7.80 (dd, $J$ = 3.5, 15.4 Hz, 2H, 2′- and 3′-H), 7.95 (s, 1H, 1-H), 9.77 (bs, 1H, 4″-OH), 12.69 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta_C$ 11.2 (C-18), 22.7 ($CH_2$), 25.8 ($CH_2$), 26.3 ($CH_2$), 29.5 ($CH_2$), 29.9 ($CH_2$), 36.6 ($CH_2$), 38.4 (CH), 42.7 (C-13), 43.4 (CH), 49.5 (CH), 55.9 (3″-$OCH_3$), 80.0 (C-17), 113.3 (C-2″), 115.8 (C-5″), 117.0 (C-2′), 117.9 (C-4), 118.4 (C-2), 123.8 (C-6″), 126.1 (C-1″), 126.9 (C-1), 131.4 (C-10), 145.7 (C-3′), 146.4 (C-5), 147.9 (C-4″), 150.1 (C-3″), 159.9 (C-3), 193.2 (C-1′); ESI-MS 447.0 [M−H]$^-$.

*2.1.2.9. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(3″-hydroxy-4″-methoxyphenyl)-prop-2′-en-1′-one **(2i)**.* According to the general procedure, isovanillin (228 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 20:80 to afford **2i** (265 mg, 59 %) as a yellow solid. Mp 221–223 °C; anal. calcd. for $C_{28}H_{32}O_5$ C, 74.98; H, 7.19; found C, 74.88; H, 7.10; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.69 (s, 3H, 18-$H_3$), 1.08–1.15 (m, 1H), 1.18–1.46 (overlapping m, 6H), 1.54–1.64 (m, 1H), 1.75–1.83 (m, 1H), 1.84–1.95 (m, 2H), 2.09–2.18 (m, 1H), 2.53–2.60 (m, 1H), 2.72–2.88 (m, 2H, 6-$H_2$), 3.54 (td, $J$ = 4.2, 8.4 Hz, 1H, 17-H), 3.85 (s, 3H, 4″-$OCH_3$), 4.50 (d, $J$ = 4.8 Hz, 1H, 17-OH), 6.66 (s, 1H, 4-H), 7.01 (d, $J$ = 8.4 Hz, 1H, 5″-H), 7.31 (dd, $J$ = 2.1, 8.3 Hz, 1H, 6″-H), 7.39 (d, $J$ = 2.1 Hz, 1H, 2″-H), 7.68–7.80 (dd-like m, 2H, 2′- and 3′-H), 7.94 (s, 1H, 1-H), 9.20 (s, 1H, 3″-OH), 12.60 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta_C$ 11.2 (C-18), 22.7 ($CH_2$), 25.8 ($CH_2$), 26.3 ($CH_2$), 29.5 ($CH_2$), 29.9 ($CH_2$), 36.5 ($CH_2$), 38.4 (CH), 42.7 (C-13), 43.4 (CH), 49.5 (CH), 55.7 (4″-$OCH_3$), 80.0 (C-17), 111.9 (C-5″), 114.9 (C-2″), 117.0 (C-4), 118.4 (C-2), 118.7 (C-2′), 122.8 (C-6″), 126.9 (C-1), 127.5 (C-1″), 131.5 (C-10), 145.2 (C-3′), 146.5 (C-3″), 146.7 (C-5), 150.6 (C-4″), 159.9 (C-3), 193.1 (C-1′); ESI-MS 449.2 [M + H]$^+$.

*2.1.2.10. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(3″,4″-dihydroxyphenyl)-prop-2′-en-1′-one **(2j)**.* According to the general procedure, 3,4-dihydroxybenzaldehyde (207 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ / AcOH = 50:50:0.5 to afford **2j** (274 mg, 63 %) as a yellow solid. Mp 212–214 °C; anal. calcd. for $C_{27}H_{30}O_5$ C, 74.63; H, 6.96; found C, 74.55; H, 6.88; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.69 (s, 3H, 18-$H_3$), 1.08–1.47 (overlapping m, 7H), 1.54–1.64 (m, 1H), 1.75–1.83 (m, 1H), 1.89–1.95 (m, 2H), 2.10–2.19 (m, 1H), 2.53–2.59 (m, 1H), 2.74–2.87 (m, 2H, 6-$H_2$), 3.54 (t, $J$ = 8.5 Hz, 1H, 17-H), 4.51 (bs, 1H, 17-OH), 6.66 (s, 1H, 4-H), 6.82 (d, $J$ = 8.1 Hz, 1H, 5″-H), 7.23 (dd, $J$ = 2.1, 8.3 Hz, 1H, 6″-H), 7.32 (d, $J$ = 2.1 Hz, 1H, 2″-H), 7.70 (bs, 2H, 2′- and 3′-H), 7.93 (s, 1H, 1-H), 9.44 (bs, 2H, 3″- and 4″-OH), 12.68 (bs, 1H, 3-OH); $^{13}$C NMR (126 MHz, DMSO): $\delta_C$ 11.2 (C-18), 22.7 ($CH_2$), 25.8 ($CH_2$), 26.3 ($CH_2$), 29.5 ($CH_2$), 29.9 ($CH_2$), 36.5 ($CH_2$), 38.4 (CH), 42.7 (C-13), 43.4 (CH), 49.5 (CH), 80.0 (C-17), 115.73 and 115.75 (2C, C-2″ and C-5″), 117.0 (C-2′), 117.4 (C-4), 118.4 (C-2), 122.9 (C-6″), 126.1 (C-1″), 126.8 (C-1), 131.5 (C-10), 145.6 (C-5), 145.7 (C-3′), 146.4 (C-3″), 149.2 (C-4″), 159.9 (C-3), 193.1 (C-1′); ESI-MS 433.0 [M−H]$^-$.

*2.1.2.11. (E)-1′-(3,17β-dihydroxyestra-1,3,5(10)-trien-2-yl)-3′-(3″,4″-dimethoxyphenyl)-prop-2′-en-1′-one **(2k)**.* According to the general procedure, 3,4-dimethoxybenzaldehyde (249 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 20:80 to afford **2k** (254 mg, 55 %) as a yellow solid. Mp 204–206 °C; anal. calcd. for $C_{29}H_{34}O_5$ C, 75.30; H, 7.41; found C, 75.31; H, 7.49; $^1$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.81 (s, 3H, 18-$H_3$), 1.17–1.29 (m, 1H), 1.29–1.55 (overlapping m, 5H), 1.54–1.66 (m, 1H), 1.67–1.77 (m, 1H), 1.86–1.94 (m, 1H), 2.01 (dt, $J$ = 3.3, 12.5 Hz, 1H), 2.09–2.24 (overlapping m, 2H), 2.34–2.42 (m, 1H), 2.84–2.94 (m, 2H, 6-$H_2$), 3.75 (t, $J$ = 8.6 Hz, 1H, 17-H), 3.95 (two overlapping s, 6H, 3″-$OCH_3$ and 4″-$OCH_3$), 6.73 (s, 1H, 4-H), 6.93 (d, $J$ = 8.3 Hz, 1H, 5″-H), 7.14 (d, $J$ = 1.9 Hz, 1H, 2″-H), 7.31 (dd, $J$ = 2.0, 8.4 Hz, 1H, 6″-H), 7.47 (d, $J$ = 15.3 Hz, 1H, 2′-H), 7.77 (s, 1H, 1-H), 7.85 (d, $J$ = 15.3 Hz, 1H, 3′-H), 12.67 (s, 1H, 3-OH); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.6 ($CH_2$), 27.0 ($CH_2$), 30.1 ($CH_2$), 30.8 ($CH_2$), 36.7 ($CH_2$), 38.8 (CH), 43.3 (C-13), 43.8 (CH), 50.2 (CH), 56.2 and 56.3 (2C, 3″-$OCH_3$ and 4″-$OCH_3$), 82.0 (C-17), 111.41 and 111.44 (C-2″ and C-5″), 117.9 (C-4), 118.3 (C-2), 118.4 (C-2′), 122.9 (C-6″), 126.2 (C-1), 128.0 (C-1″), 131.5 (C-10), 145.2 (C-3′), 147.2 (C-5), 149.4 (C-4″), 151.8 (C-3″), 161.3 (C-3), 193.4 (C-1′); ESI-MS 463.2 [M + H]$^+$.

*2.1.3. General procedure for the synthesis of flavones **(3a-k)***

To a solution of the chalcone (0.50 mmol) in DMSO (5 mL) elemental iodine (6 mg) was added. The resulting brown mixture was stirred at 130 °C for 1 h. After completion, the reaction mixture was poured into water, and the resulting brown precipitate was filtered off and dried. The crude product was purified by column chromatography.

*2.1.3.1. 17β-hydroxy-2′-furyl-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one **(3a)**.* According to the general procedure, **2a** (196 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 50:50 to afford **3a** (137 mg, 70 %) as a white solid. Mp 292–294 °C; anal. calcd. for $C_{25}H_{26}O_4$ C, 76.90; H, 6.71; found C, 76.85; H, 6.62; $^1$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.80 (s, 3H, 18-$H_3$), 1.18–1.68 (overlapping m, 7H), 1.67–1.77 (m, 1H), 1.90–1.99 (m, 1H), 2.02 (dt, $J$ = 3.3, 12.5 Hz, 1H), 2.08–2.19 (m, 1H), 2.24–2.33 (m, 1H), 2.49–2.57 (m, 1H), 2.93–3.09

(m, 2H, 6-H$_2$), 3.76 (t, $J$ = 8.5 Hz, 1H, 17-H), 6.59 (dd, $J$ = 1.7, 3.4 Hz, 1H, 4″-H), 6.69 (s, 1H, 3′-H), 7.08 (d, $J$ = 3.5 Hz, 1H, 3″-H), 7.21 (s, 1H, 4-H), 7.61 (d, $J$ = 1.7 Hz, 1H, 5″-H), 8.10 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta_C$ 11.2 (C-18), 23.3 (CH$_2$), 26.4 (CH$_2$), 27.0 (CH$_2$), 30.0 (CH$_2$), 30.6 (CH$_2$), 36.7 (CH$_2$), 38.5 (CH), 43.4 (C-13), 44.3 (CH), 50.4 (CH), 81.9 (C-17), 105.5 (C-3′), 112.6 (C-4″), 112.7 (C-3″), 117.2 (C-4), 122.08 (C-2), 122.13 (C-1), 138.7 (C-10), 144.6 (C-2″), 145.7 (C-5″), 146.8 (C-5), 154.2 (C-3), 155.0 (C-2′), 178.1 (C-4′); ESI-MS 391.2 [M + H]$^+$.

*2.1.3.2. 17β-hydroxy-2′-thiophenyl-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3b**).* According to the general procedure, **2b** (204 mg) was used. The crude product was purified with EtOAc / CH$_2$Cl$_2$ = 50:50 to afford **3b** (177 mg, 87 %) as a white solid. Mp 268–270 °C; anal. calcd. for $C_{25}H_{26}O_3S$ C, 73.86; H, 6.45; found C, 73.77; H, 6.41; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 0.80 (s, 3H, 18-H$_3$), 1.18–1.77 (overlapping m, 8H), 1.90–1.98 (m, 1H), 2.04 (dt, $J$ = 3.4, 12.6 Hz, 1H), 2.07–2.18 (m, 1H), 2.28 (td, $J$ = 4.4, 11.2 Hz, 1H), 2.48–2.56 (m, 1H), 2.92–3.08 (m, 2H, 6-H$_2$), 3.76 (t, $J$ = 8.5 Hz, 1H, 17-H), 6.64 (s, 1H, 3′-H), 7.16 (dd, $J$ = 3.8, 5.0 Hz, 1H, 4″-H), 7.23 (s, 1H, 4-H), 7.55 (d, $J$ = 4.9 Hz, 1H, 3″-H), 7.68 (dd, $J$ = 1.1, 3.7 Hz, 1H, 5″-H), 8.09 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta_C$ 11.2 (C-18), 23.3 (CH$_2$), 26.4 (CH$_2$), 26.9 (CH$_2$), 30.0 (CH$_2$), 30.5 (CH$_2$), 36.7 (CH$_2$), 38.5 (CH), 43.4 (C-13), 44.3 (CH), 50.4 (CH), 81.8 (C-17), 106.1 (C-3′), 117.2 (C-4), 121.8 (C-2), 122.0 (C-1), 128.2 (C-3″), 128.5 (C-4″), 130.1 (C-5″), 135.6 (C-2″), 138.8 (C-10), 144.7 (C-5), 154.3 (C-3), 158.8 (C-2′), 178.2 (C-4′); ESI-MS 407.2 [M + H]$^+$.

*2.1.3.3. 17β-hydroxy-2′-phenyl-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3c**).* According to the general procedure, **2c** (200 mg) was used. The crude product was purified with EtOAc / CH$_2$Cl$_2$ = 50:50 to afford **3c** (182 mg, 91 %) as a white solid. Mp 244–246 °C; anal. calcd. for $C_{27}H_{28}O_3$ C, 80.97; H, 7.05; found C, 80.90; H, 7.16; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 0.81 (s, 3H, 18-H3), 1.19–1.27 (m, 1H), 1.29–1.58 (overlapping m, 5H), 1.61–1.67 (m, 1H), 1.69–1.76 (m, 1H), 1.91–2.00 (m, 1H), 2.00–2.07 (m, 1H), 2.08–2.19 (m, 1H), 2.26–2.35 (m, 1H), 2.49–2.59 (m, 1H), 2.94–3.10 (m, 2H, 6-H2), 3.77 (t, $J$ = 8.6 Hz, 1H, 17-H), 6.78 (s, 1H, 3′-H), 7.28 (s, 1H, 4-H), 7.47–7.55 (m, 3H, 3″-, 4″- and 5″-H), 7.88–7.94 (m, 2H, 2″- and 6″-H), 8.12 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta_C$ 11.1 (C-18), 23.2 (CH$_2$), 26.3 (CH$_2$), 26.8 (CH$_2$), 29.9 (CH$_2$), 30.5 (CH$_2$), 36.6 (CH$_2$), 38.4 (CH), 43.3 (C-13), 44.2 (CH), 50.3 (CH), 81.8 (C-17), 107.3 (C-3′), 117.2 (C-4), 121.7 (C-2), 121.9 (C-1), 126.2 (2C, C-2″ and C-6″), 129.0 (2C, C-3″ and C-5″), 131.4 (C-4″), 132.1 (C-1″), 138.7 (C-10), 144.6 (C-5), 154.5 (C-3), 163.0 (C-2′), 178.6 (C-4′); ESI-MS 401.2 [M + H]$^+$.

*2.1.3.4. 17β-hydroxy-2′-(4″-methylphenyl)-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3d**).* According to the general procedure, **2d** (208 mg) was used. The crude product was purified with EtOAc / CH$_2$Cl$_2$ = 50:50 to afford **3d** (164 mg, 79 %) as a white solid. Mp 256–258 °C; anal. calcd. for $C_{28}H_{30}O_3$ C, 81.13; H, 7.29; found C, 81.16; H, 7.29; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 0.80 (s, 3H, 18-H$_3$), 1.29–1.56 (overlapping m, 5H), 1.58–1.78 (overlapping m, 3H), 1.90–1.99 (m, 1H), 2.03 (dt, $J$ = 3.4, 12.6 Hz, 1H), 2.07–2.19 (m, 1H), 2.29 (td, $J$ = 4.3, 11.1 Hz, 1H), 2.43 (s, 3H, 4″-CH$_3$), 2.49–2.58 (m, 1H), 2.95–3.06 (m, 2H, 6-H$_2$), 3.76 (t, $J$ = 8.6 Hz, 1H, 17-H), 6.74 (s, 1H, 3′-H), 7.27 (s, 1H, 4-H), 7.31 (d, $J$ = 8.0 Hz, 2H, 2″- and 6″-H), 7.80 (d, $J$ = 8.1 Hz, 2H, 3″- and 5″-H), 8.11 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta_C$ 11.2 (C-18), 21.6 (4″-CH$_3$), 23.3 (CH$_2$), 26.4 (CH$_2$), 27.0 (CH$_2$), 30.0 (CH$_2$), 30.6 (CH$_2$), 36.7 (CH$_2$), 38.5 (CH), 43.4 (C-13), 44.3 (CH), 50.4 (CH), 81.9 (C-17), 106.8 (C-3′), 117.3 (C-4), 121.8 (C-2), 122.0 (C-1), 126.3 (2C, C-3″ and C-5″), 129.4 (C-1″), 129.8 (2C, C-2″ and C-6″), 138.7 (C-10), 142.1 (C-4″), 144.5 (C-5), 154.6 (C-3), 163.3 (C-2′), 178.8 (C-4′); ESI-MS 415.2 [M + H]$^+$.

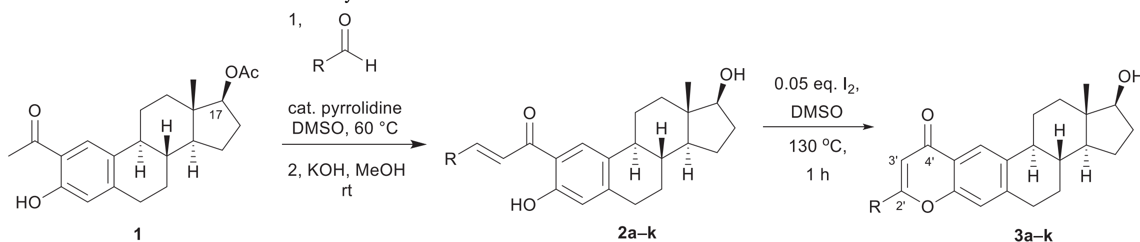
*2.1.3.5. 17β-hydroxy-2′-(4″-bromophenyl)-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3e**).* According to the general procedure, **2e** (241 mg) was used. The crude product was purified with EtOAc / CH$_2$Cl$_2$ = 50:50 to afford **3e** (213 mg, 89 %) as a white solid. Mp 263–265 °C; anal. calcd. for $C_{27}H_{27}BrO_3$ C, 67.64; H, 5.68; found C, 67.54; H, 5.54; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 0.80 (s, 3H, 18-H$_3$), 1.17–1.78 (overlapping m, 8H), 1.92–1.99 (m, 1H), 2.02 (dd, $J$ = 3.4, 12.8 Hz, 1H), 2.08–2.19 (m, 1H), 2.30 (td, $J$ = 4.3 Hz, 11.2 Hz, 1H), 2.49–2.57 (m, 1H), 2.94–3.11 (m, 2H, 6-H$_2$), 3.72–3.80 (m, 1H, 17-H), 6.74 (s, 1H, 3′-H), 7.27 (s, 1H, 4-H), 7.64 (d, $J$ = 8.6 Hz, 2H, 3″- and 5″-H), 7.76 (d, $J$ = 8.7 Hz, 2H, 2″- and 6″-H), 8.11 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta_C$ 11.2 (C-18), 23.3 (CH$_2$), 26.4 (CH$_2$), 26.9 (CH$_2$), 30.0 (CH$_2$), 30.6 (CH$_2$), 36.7 (CH$_2$), 38.5 (CH), 43.4 (C-13), 44.3 (CH), 50.4 (CH), 81.9 (C-17), 107.6 (C-3′), 117.3 (C-4), 121.8 (C-2), 122.1 (C-1), 126.2 (C-4″), 127.8 (2C, C-2″ and C-6″), 131.2 (C-1″), 132.4 (2C, C-3″ and C-5″), 139.0 (C-10), 144.9 (C-5), 154.5 (C-3), 162.0 (C-2′), 178.5 (C-4′); ESI-MS 479.1 [M + H]$^+$, 481.1 [M + H]$^+$.

*2.1.3.6. 17β-hydroxy-2′-(4″-hydroxyphenyl)-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3f**).* According to the general procedure, **2f** (209 mg) was used. The crude product was purified with MeOH / CH$_2$Cl$_2$ = 5:95 to afford **3f** (140 mg, 67 %) as a white solid. Mp > 300 °C (decomposes); anal. calcd. for $C_{27}H_{28}O_4$ C, 77.86; H, 6.78; found C, 77.74; H, 6.71; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.67 (s, 3H, 18-H$_3$), 1.06–1.53 (overlapping m, 7H), 1.54–1.64 (m, 1H), 1.79–1.93 (overlapping m, 3H), 2.16–2.25 (m, 1H), 2.30–2.39 (dt, $J$ = 3.5, 13.4 Hz, 1H), 2.87–3.02 (m, 2H, 6-H$_2$), 3.52 (td, $J$ = 4.7, 8.4 Hz, 1H, 17-H), 4.50 (d, $J$ = 4.8 Hz, 1H, 17-OH), 6.77 (s, 1H, 3′-H), 6.93 (d, $J$ = 8.7 Hz, 2H, 3″-H and 5″-H), 7.38 (s, 1H, 4-H), 7.85 (s, 1H, 1-H), 7.91 (d, $J$ = 8.7 Hz, 2H, 2″- and 6″-H), 10.26 (s, 1H, 4″-OH); $^{13}$C NMR (126 MHz, DMSO- $d_6$): $\delta_C$ 11.1 (C-18), 22.7 (CH$_2$), 25.9 (CH$_2$), 26.3 (CH$_2$), 29.1 (CH$_2$), 29.8 (CH$_2$), 36.4 (CH$_2$), 37.9 (CH), 42.7 (C-13), 43.4 (CH), 49.6 (CH), 79.9 (C-17), 104.5 (C-3′), 115.9 (2C, C-3″ and C-5″), 117.2 (C-4), 120.5 (C-1), 121.0 (C-2), 121.8 (C-1″), 128.1 (2C, C-2″ and C-6″), 138.1 (C-10), 144.1 (C-5), 153.6 (C-3), 160.8 (C-4″), 162.6 (C-2′), 176.8 (C-4′); ESI-MS 417.2 [M + H]$^+$.

*2.1.3.7. 17β-hydroxy-2′-(4″-methoxyphenyl)-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3g**).* According to the general procedure, **2g** (216 mg) was used. The crude product was purified with EtOAc / CH$_2$Cl$_2$ = 50:50 to afford **3g** (146 mg, 68 %) as a white solid. Mp 226–228 °C; anal. calcd. for $C_{28}H_{30}O_4$ C, 78.11; H, 7.02; found C, 78.08; H, 7.01; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 0.81 (s, 3H, 18-H$_3$), 1.17–1.57 (overlapping m, 6H), 1.58–1.78 (m, 2H), 1.91–1.99 (m, 1H), 1.99–2.07 (m, 1H), 2.08–2.19 (m, 1H), 2.29 (td, $J$ = 4.3, 11.1 Hz, 1H), 2.49–2.58 (m, 1H), 2.94–3.08 (m, 2H, 6-H$_2$), 3.76 (t, $J$ = 8.5 Hz, 1H, 17-H), 3.88 (s, 3H, 4″-OCH$_3$), 6.69 (s, 1H, 3′-H), 7.01 (d, $J$ = 8.8 Hz, 2H, 3″- and 5″-H), 7.25 (s, 1H, 4-H), 7.86 (d, $J$ = 8.7 Hz, 2H, 2″- and 6″-H), 8.11 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta_C$ 11.2 (C-18), 23.3 (CH$_2$), 26.4 (CH$_2$), 27.0 (CH$_2$), 30.0 (CH$_2$), 30.7 (CH$_2$), 36.8 (CH$_2$), 38.6 (CH), 43.4 (C-13), 44.3 (CH), 50.5 (CH), 55.6 (4″-OCH$_3$), 81.9 (C-17), 106.1 (C-3′), 114.6 (2C, C-3″ and C-5″), 117.2 (C-4), 121.9 (C-2), 122.0 (C-1), 124.6 (C-1″), 128.1 (2C, C-2″ and C-6″), 138.7 (C-10), 144.4 (C-5), 154.6 (C-3), 162.5 (C-2′), 163.2 (C-4″), 178.6 (C-4′); ESI-MS 431.2 [M + H]$^+$.

*2.1.3.8. 17β-hydroxy-2′-(4″-hydroxy-3″-methoxyphenyl)-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3h**).* According to the general procedure, **2h** (224 mg) was used. The crude product was purified with MeOH / CH$_2$Cl$_2$ = 5:95 to afford **3h** (176 mg, 79 %) as a white solid. Mp > 300 °C (decomposes); anal. calcd. for $C_{28}H_{30}O_5$ C, 75.31; H, 6.77; found C, 75.23; H, 6.70; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.67 (s, 3H, 18-H$_3$), 1.07–1.53 (overlapping m, 7H), 1.54–1.64 (m, 1H), 1.80–1.94 (m, 3H), 2.21 (td, $J$ = 4.2, 10.8 Hz, 1H), 2.34 (dd, $J$ = 4.1, 9.5 Hz, 1H), 2.86–3.03 (m, 2H, 6-H$_2$), 3.53 (t, $J$ = 8.5 Hz, 1H, 17-H), 3.88 (s, 3H, 3″-OCH$_3$), 4.51 (bs, 1H, 17-OH), 6.84 (s, 1H, 3′-H), 6.91 (d-like m, 1H of

**Table 1**
Synthesis of estradiol-chalcone and estradiol-flavone hybrids.




| Entry | R | Chalcone | Yield[a] (%) | Flavone | Yield[a] (%) |
|---|---|---|---|---|---|
| 1 | O | **2a** | 74 | **3a** | 70 |
| 2 | S | **2b** | 69 | **3b** | 87 |
| 3 | | **2c** | 78 | **3c** | 91 |
| 4 | | **2d** | 86 | **3d** | 79 |
| 5 | Br | **2e** | 58 | **3e** | 89 |
| 6 | OH | **2f** | 68 | **3f** | 67 |
| 7 | OMe | **2g** | 79 (77[b]) | **3g** | 68 |
| 8 | OH, OMe | **2h** | 60 | **3h** | 79 |
| 9 | OMe, OH | **2i** | 59 | **3i** | 81 |
| 10 | OH, OH | **2j** | 63 | **3j** | 66 |
| 11 | OMe, OMe | **2k** | 55 | **3k** | 76 |

[a]Yields after chromatographic purification; [b]Previously prepared by Wang et al. *via* Claisen-Schmidt condensation [25].

ring), 7.41 (s, 1H, 4-H), 7.49–7.56 (m, 2H of ring), 7.85 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, DMSO): $\delta_C$ 11.1 (C-18), 22.7 ($CH_2$), 25.9 ($CH_2$), 26.3 ($CH_2$), 29.0 ($CH_2$), 29.8 ($CH_2$), 36.4 ($CH_2$), 37.9 (CH), 42.7 (C-13), 43.4 (CH), 49.6 (CH), 55.8 (4″-$OCH_3$), 79.9 (C-17), 104.4 (C-3′), 109.9 (C-2″), 115.9 (C-5″), 117.2 (C-4), 120.1 (C-6″), 120.5 (C-1), 121.0 (C-2), 138.0 (C-10), 144.0 (C-5), 148.2 (C-4″), 151.7 (C) 153.6 (C-3), 162.7 (C-2′), 169.3 (C), 176.8 (C-4′); due to its poor solubility in conventional deuterated solvents, a $^{13}$C spectrum with a satisfying signal/noise ratio could not be obtained despite our best efforts, thus the assignation is somewhat ambiguous; ESI-MS 447.2 [M + H]$^+$.

*2.1.3.9. 17β-hydroxy-2′-(3″-hydroxy-4″-methoxyphenyl)-4′H-pyrano [5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3i**).* According to the general procedure, **2i** (224 mg) was used. The crude product was purified with MeOH / $CH_2Cl_2$ = 5:95 to afford **3i** (181 mg, 81 %) as a white solid. Mp > 260 °C (decomposes); anal. calcd. for $C_{28}H_{30}O_5$ C, 75.31; H, 6.77; found C, 75.36; H, 6.78; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.66 (s, 3H, 18-$H_3$), 1.05–1.52 (overlapping m, 7H), 1.53–1.63 (m, 1H), 1.79–1.93 (overlapping m, 3H), 2.19 (td, $J$ = 4.1, 10.9 Hz, 1H), 2.28–2.38 (m, 1H), 2.87–3.02 (m, 2H, 6-$H_2$), 3.52 (t, $J$ = 8.5 Hz, 1H, 17-H), 3.86 (s, 3H, 4″-$OCH_3$), 4.50 (s, 1H, 17-OH), 6.72 (s, 1H, 3′-H), 7.08 (d, $J$ = 8.6 Hz, 1H, 5″-H), 7.37 (s, 1H, 4-H), 7.42 (d, $J$ = 2.3 Hz, 1H, 2″-H), 7.51 (dd, $J$ = 2.3, 8.5 Hz, 1H, 6″-H), 7.84 (s, 1H, 1-H), 9.41 (s, 1H, 3″-OH); $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta_C$ 11.1 (C-18), 22.7 ($CH_2$), 25.9 ($CH_2$), 26.2 ($CH_2$), 29.1 ($CH_2$), 29.8 ($CH_2$), 36.4 ($CH_2$), 37.8 (CH), 42.7 (C-13), 43.4 (CH), 49.6 (CH), 55.7 (4″-$OCH_3$), 79.9 (C-17), 105.1 (C-3′), 112.1 (C-2″), 112.9 (C-5″), 117.1 (C-4), 118.3 (C-6″), 120.5 (C-1), 121.0 (C-2), 123.6 (C-1″), 138.2 (C-10), 144.2 (C-5), 146.8 (C-3″), 150.8 (C-4″), 153.6 (C-3), 162.4 (C-2′), 176.7 (C-4′); ESI-MS 447.2 [M + H]$^+$, 469.1 [M + Na]$^+$.

*2.1.3.10. 17β-hydroxy-2′-(3″,4″-dihydroxyphenyl)-4′H-pyrano[5′,6′-2,3] estra-1,3,5(10)-trien-4′-one (**3j**).* According to the general procedure, **2j** (217 mg) was used. The crude product was purified with MeOH / $CH_2Cl_2$ / AcOH = 5:95:1 to afford **3j** (143 mg, 66 %) as an off-white solid. Mp > 300 °C (decomposes); anal. calcd. for $C_{27}H_{28}O_5$ C, 74.98H, 6.53; found C, 75.08, H, 6.61; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 0.67 (s, 3H, 18-$H_3$), 1.07–1.53 (overlapping m, 7H), 1.54–1.64 (m, 1H), 1.79–1.92 (overlapping m, 3H), 2.21 (td, $J$ = 4.2, 10.9 Hz, 1H), 2.31–2.38 (m, 1H), 2.87–3.03 (m, 2H, 6-$H_2$), 3.52 (t, $J$ = 8.5 Hz, 1H, 17-H), 4.49 (bs, 1H, 17-OH), 6.65 (s, 1H, 3′-H), 6.89 (d, $J$ = 8.9 Hz, 1H, 5″-H), 7.36 (s, 1H, 4-H), 7.37–7.43 (m, 2H, 2″- and 6″-H), 7.85 (s, 1H, 1-H), 9.62 (bs, 2H, 3″- and 4″-OH); $^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta_C$ 11.1 (C-13), 22.7 ($CH_2$), 25.9 ($CH_2$), 26.3 ($CH_2$), 29.1 ($CH_2$), 29.8 ($CH_2$), 36.4 ($CH_2$), 37.9 (CH), 42.7 (C-13), 43.4 (CH), 49.6 (CH), 79.9 (C-17), 104.5 (C-3′), 113.2 (C-2″), 115.9 (C-5″), 117.1 (C-4), 118.6 (C-6″), 120.5 (C-1), 121.0 (C-2), 122.1 (C-1″), 138.1 (C-10), 144.1 (C-5), 145.7 (C-3″), 149.3 (C-4″), 153.6 (C-3), 162.8 (C-2′), 176.7 (C-4′); ESI-MS 433.2 [M + H]$^+$.

*2.1.3.11. 17β-hydroxy-2′-(3″,4″-dimethoxyphenyl)-4′H-pyrano[5′,6′-2,3]estra-1,3,5(10)-trien-4′-one (**3 k**).* According to the general procedure, **2k** (231 mg) was used. The crude product was purified with EtOAc / $CH_2Cl_2$ = 50:50 to afford **3k** (175 mg, 76 %) as a white solid. Mp 238–240 °C; anal. calcd. for $C_{29}H_{32}O_5$ C, 75.63; H, 7.00; found C, 75.50; H, 6.91; $^1$H NMR (500 MHz, $CDCl_3$): $\delta_H$ 0.80 (s, 3H, 18-$H_3$), 1.18–1.28 (m, 1H), 1.29–1.57 (overlapping m, 5H), 1.57–1.77 (m, 2H), 1.91–1.99 (m, 1H), 2.04 (dt, $J$ = 3.4, 12.5 Hz, 1H), 2.08–2.19 (m, 1H), 2.30 (td, $J$ = 4.3, 11.1 Hz, 1H), 2.49–2.58 (m, 1H), 2.94–3.08 (m, 2H, 6-$H_2$), 3.76 (t, $J$ = 8.5 Hz, 1H, 17-H), 3.96 (s, 3H, 4″-$OCH_3$), 3.98 (s, 3H, 3″-$OCH_3$), 6.71 (s, 1H, 3′-H), 6.97 (d, $J$ = 8.5 Hz, 1H, 5″-H), 7.27 (s, 1H, 4-H), 7.38 (d, $J$ = 2.1 Hz, 1H, 2″-H), 7.54 (dd, $J$ = 2.1, 8.5 Hz, 1H, 6″-H), 8.11 (s, 1H, 1-H); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta_C$ 11.2 (C-18), 23.3 ($CH_2$), 26.4 ($CH_2$), 27.0 ($CH_2$), 30.0 ($CH_2$), 30.6 ($CH_2$), 36.7 ($CH_2$), 38.6 (CH), 43.4 (C-13), 44.3 (CH), 50.4 (CH), 56.2 (2C, 3″- and 4″-$OCH_3$), 81.9 (C-17), 106.3 (C-3′), 109.0 (C-2″), 111.3 (C-5″), 117.3 (C-4), 120.0 (C-6″), 121.8 (C-2), 122.0 (C-1), 124.7 (C-1″), 138.7 (C-10), 144.5 (C-5), 149.4 (C-3″), 152.1 (C-4″), 154.6 (C-3), 163.1 (C-2′), 178.7 (C-4′); ESI-MS 461.2 $[M + H]^+$.

#### 2.1.4. Synthesis of 2-(3′-phenyl-1′H-pyrazol-5′-yl)-3,17β-dihydroxyestra-1,3,5(10)-triene (4c)

To a suspension of **3c** (0.50 mmol, 200 mg) in abs. EtOH (4 mL) 50 % hydrazine hydrate (0.16 mL, 5 equiv.) was added. The suspension was then irradiated for 1 h at 120 °C, after which an additional 0.16 mL of hydrazine hydrate was added, and the pale yellow solution was irradiated at 120 °C for one more hour. The resulting homogenous mixture was then poured onto water and extracted with ethyl acetate. The combined organic phases were washed with brine and dried over anhydrous $Na_2SO_4$. The crude product was purified by column chromatography, using EtOAc / $CH_2Cl_2$ = 20:80 as eluent to afford **4c** (161 mg, 78 %) as a white solid. Mp > 280 °C (decomposes); anal. calcd. for $C_{27}H_{30}N_2O_2$ C, 78.23; H, 7.29; found C, 78.36; H, 7.41; this compound is a mixture of tautomers $T_{OH-N}$ (a) and $T_{NH-O}$ (b) with a ratio of roughly 2:1; $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta_H$ 0.69 (s, 3H, 18-$CH_3$), 1.11–1.44 (overlapping m, 7H), 1.55–1.64 (m, 1H), 1.76–1.76–1.83 (m, 1H), 1.84–1.94 (m, 2H), 2.09–2.19 (m, 1H), 2.43–2.53 (m, 1H), 2.70–2.80 (m, 2H, 6-$H_2$), 3.50–3.58 (m, 1H, 17-H), 4.50 (d, $J$ = 4.9 Hz, 1H, 17-OH), 6.60 (bs, a) and 6.65 (bs, b): 4′-H, 7.14 (bs, 4-H, b), 7.29–7.31 (*t*-like m, b, 4″-H), 7.37–7.44 (m, 3″-H, 5″-H (b) and 4-H (a) and 4″-H (a)), 7.50 (t, $J$ = 7.6 Hz, 3″-H, 5″-H (a)), 7.57 (bs, b) and 7.65 (bs, a): 1-H, 7.86 (d, $J$ = 7.5 Hz, 2H, 2″-H and 6″-H), 9.90 (s, b) and 10.64 (s, a): 3-OH, 12.77 (bs, b) and 13.53 (bs, a): NH; $^{13}$C NMR (DMSO-$d_6$, 126 MHz): $\delta_C$ 11.3 (C-18), 22.8 ($CH_2$), 26.1 ($CH_2$), 26.8 ($CH_2$), 29.0 ($CH_2$), 29.9 ($CH_2$), 36.6 ($CH_2$), 38.6 (CH), 42.8 (C-13), 43.6 (CH), 49.6 (CH), 80.1 (C-17), 99.6 (a) and 100.7 (b): C-4′, 113.6 (b) and 114.5 (a): C-2, 115.9 (a) and 123.4 (b): C-4, 124.1 (b) and 125.0 (a): C-1, 125.4 (2C, C-2″ and C-6″), 127.1 (b) and 128.6 (a): C-4″, 128.8 (b) and 129.7 (a): C-10, 129.0 (2C, C-3″ and C-5″), 131.1 (a) and 131.2 (b): C-4″, 137.3 (C-5), 141.2 (b) and 142.9 (a): C-3′, 151.7 (b) and 151.9 (a): C-5′, 153.1 (C-3); ESI-MS 415.2 $[M + H]^+$.

### 2.2. Pharmacological and statistical methods

#### 2.2.1. Cell culture

All cell lines were obtained from ATCC. DU-145, PC-3 (both prostate cancer), HeLa (cervical cancer) and MCF-7 (breast cancer) cell lines were maintained in RPMI-1640 medium (Biosera), while MRC-5 (non-cancerous fibroblast) cells were cultured in EMEM medium (Biosera). Media were supplemented with 10 % foetal bovine serum (FBS), 2 mM glutamine and 1 % penicillin–streptomycin. Cells were cultured under standard conditions in a 37 °C incubator containing 5 % $CO_2$ in 95 % humidity.

#### 2.2.2. Cell viability assay

For the cytotoxicity studies, each compound was dissolved in cell culture grade DMSO (Sigma), at 10 mM final concentration. Cells were seeded in 96-well plates with $10^4$ cells/well density, and were left to grow for 24 h. Then the estradiol-chalcone and flavone hybrids, compound **4c** as well as apigenin and estradiol were applied on cells in 2.5 μM final concentration, and cells were incubated with the test compounds for 72 h. For assessing $IC_{50}$ values, cells were treated with a serial dilution of either compound **3c**, **3d** or **3e** at 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, 7.2 8.0 μM concentration for 72 h, or with cisplatin at 20, 40, 60, 80, 330 μM concentration for 24 h. After both the primary screens as well as the experiments to determine $IC_{50}$ values, MTT assays were performed. For this, the media used for the treatments were discarded and replaced by fresh media, containing 0.5 mg/mL MTT-reagent (Sigma-Aldrich). Cells were incubated with the MTT reagent for one hour, then the resulting formazan crystals were solubilized in DMSO, and the absorbance of the samples was measured at 570 nm with Synergy HTX microplate reader (BIOTEK®). MTT assays were performed at least three times using three independent biological replicates. The viability of untreated cells was considered as 100 %.

#### 2.2.3. RNA isolation, reverse transcription and real-time qPCR

For RNA isolation, cells were seeded in 6-well plates with $10^6$ cells/well density, and left to grow for 24 h. The next day cells were treated with compound **3c**, **3d** or **3e** for 72 h. After treatment, total RNA was isolated with RNeasy® Mini Kit (QIAGEN) according to the manufacturer's recommendation. Concentration of the isolated total RNA was measured with NanoDrop ND 1000 Spectrophotometer (Thermo Fisher Scientific). From each sample, 900 ng RNA in 20 μL reaction volume was reverse transcribed with TaqMan® Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific) following the manufacturer's instructions. cDNA was diluted 5X to a final volume of 100 μL. qPCR

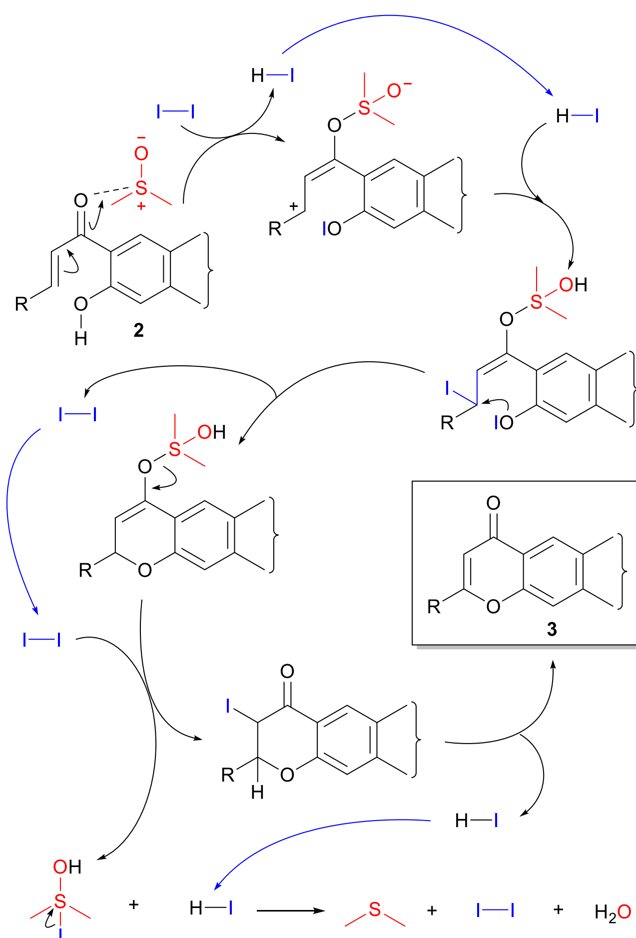
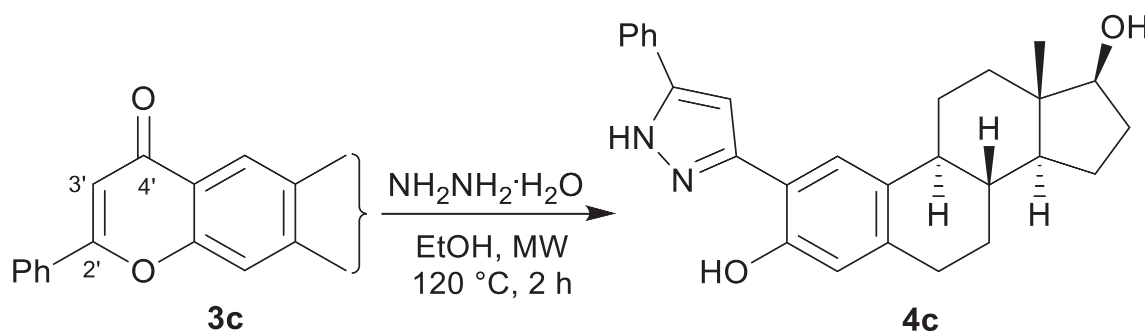



**Fig. 2.** Plausible mechanism for the $I_2$-catalyzed oxidative cyclization of 2-hydroxychalcones in DMSO.




**Scheme 1.** Conversion of flavone **3c** into 2-pyrazolyl-estradiol derivative.

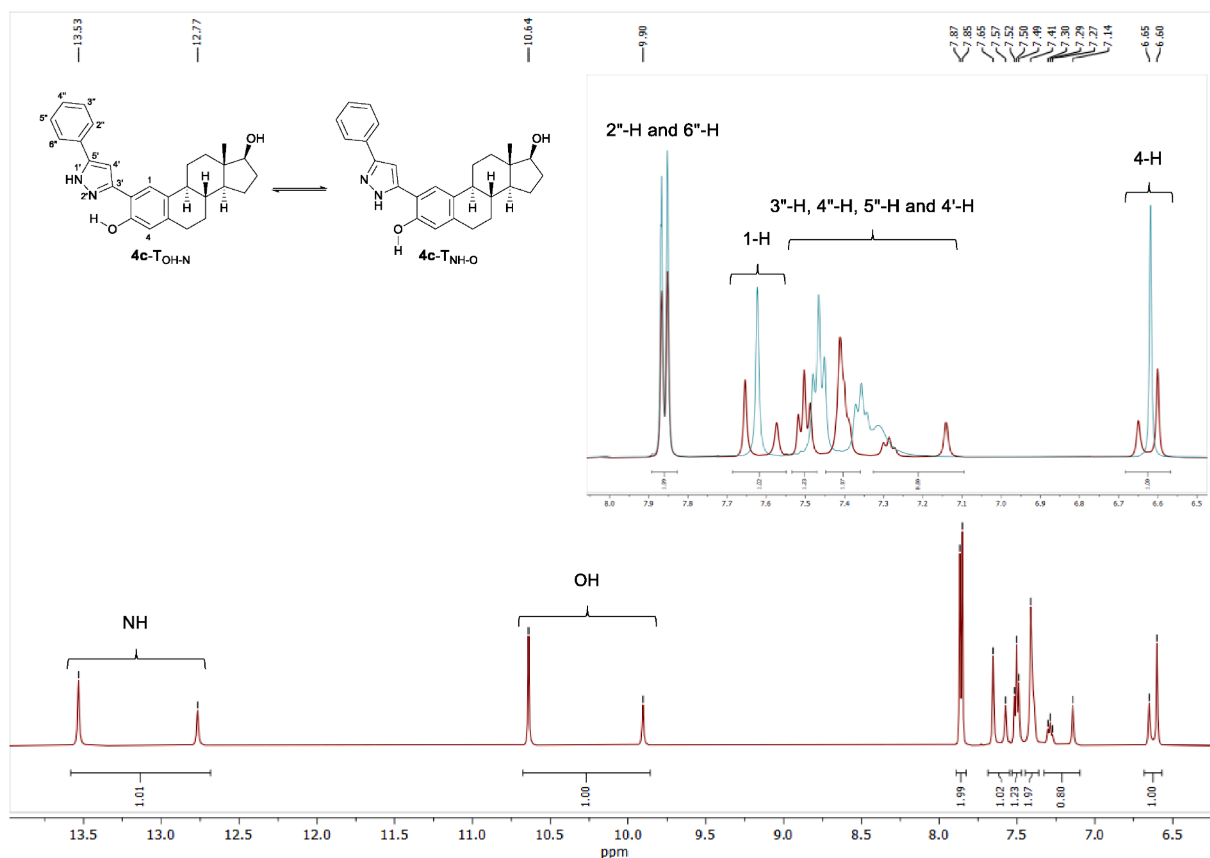



**Fig. 3.** Partial $^{1}H$ NMR spectrum of compound **4c** showing the individual proton peaks of each tautomer (big figure) and the coalescence to single peaks after equilibration (small figure at top right).

reactions were performed on PicoReal™ Real-time PCR (Thermo Fisher Scientific) using SYBR Green qPCR Master Mix (Thermo Fisher Scientific). Reactions were carried out in 10 µL reaction volume (5 µL SYBR Green, 3 µL RNase-free $H_2O$, 1 µL cDNA, 1 µL primer-mix). Relative transcript levels were determined by the ΔΔCt method, using GAPDH as the reference gene. Experiments were repeated three times with two biological replicates.

*2.2.4. Statistical analysis*

For the pharmacology related studies, data analysis, $IC_{50}$ value calculations, graphical representation of data (heat maps, dose–response curves and qPCR diagrams), statistical analysis (non-linear regression, two-way ANOVA) were carried out by GraphPad Prism 8.0.1 software. Differences between the control and treated samples were considered statistically significant, if $P < 0.05$.

## 3. Results and discussion

### *3.1. Syntheses*

For the synthesis of estradiol hybrids, 2-acetylestradiol-17β-acetate (**1**) was prepared as the starting material (Table 1) in a two-step one-pot reaction from estadiol 3-methyl ether via an $AlCl_3$-mediated Friedel-Crafts acetylation/*O*-demethylation sequence under the conditions we had previously optimized [29]. The acetyl group of **1** makes the molecule suitable for the formation of chalcones by the Claisen-Schmidt condensation with various (hetero)aryl aldehydes under basic conditions. It is worth mentioning that some similar methoxy- and chloro-substituted steroidal chalcones were previously prepared, and those containing the electron-donating OMe group in the chalcone B-ring displayed better antiproliferative action against six different human cancer cell lines than compounds with the electron-withdrawing Cl

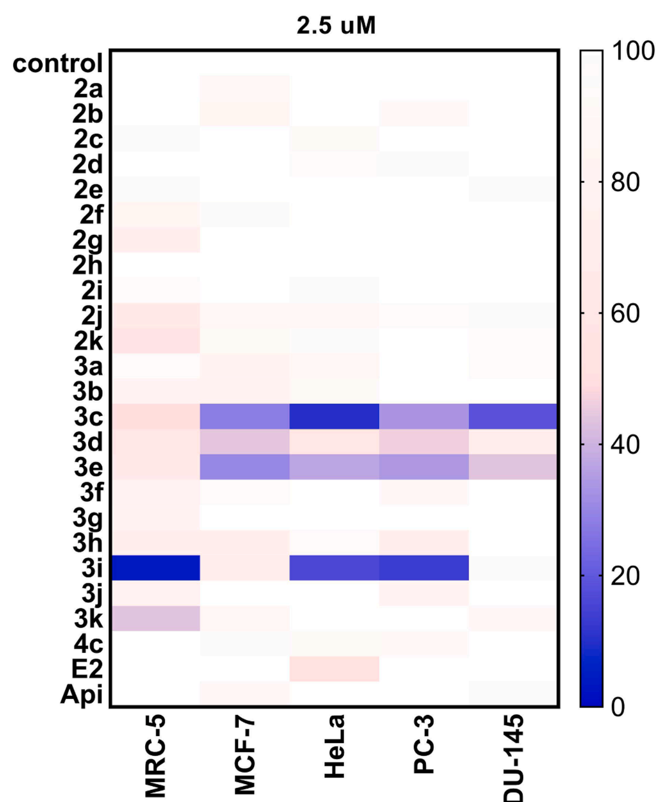



**Fig. 4.** Representative heat map of the primary cytotoxic effects of estradiol derivatives on cancerous MCF-7, HeLa, PC-3 and DU-145 cell lines as well as on non-cancerous human fibroblasts (MRC-5) (compound concentration = 2.5 μM; incubation time = 72 h). Control represents the viability of untreated cells.

**Table 2**
$IC_{50}$ values (μM ± SD) of the selected compounds and cisplatin determined on various cancer cell lines and on non-cancerous MRC-5 cells.

| Cell lines | $IC_{50}$ values (μM ± SD) | | | |
|---|---|---|---|---|
| | **3c** | **3d** | **3e** | **cisplatin** |
| MRC-5 | 10.5 ± 1.1 | 9.5 ± 1.0 | >10 | 32 ± 1.2 |
| MCF-7 | 5.6 ± 1.0 | 8.0 ± 1.0 | 7.8 ± 1.1 | 296 ± 1.1 |
| HeLa | 3.9 ± 1.1 | 6.3 ± 1.0 | 6.9 ± 1.0 | >330 |
| DU-145 | 5.6 ± 1.0 | 9.7 ± 1.1 | 6.2 ± 1.2 | 118 ± 1.1 |
| PC-3 | 6.2 ± 1.1 | 8.7 ± 1.0 | 9.5 ± 1.1 | >330 |

atoms [25]. However, the selectivity of the most active *meta*-methoxy derivative on cancer cell lines over a normal cell line was found inadequate.

Nevertheless, some additional A-ring-integrated chalcones (**2a**-**e**) have been designed by reacting **1** with heteroarylaldehydes, benzaldehyde and arylaldehydes that differ in the electron demand of their substituents on the aromatic ring, respectively (Table 1). Taking into account that several natural and synthetic chalcones contain one or more OH and/or OMe groups on their B-ring, our further aim was to extend the compound library with such derivatives (**2f**-**k**) as well. Preliminary experiments were carried out in alkaline ethanol at 40 °C for 24 h but some reactions did not lead to sufficient conversion under these classical Claisen-Schmidt conditions. Although deacetylation at C-17 occurred during the transformations in all cases due to the strongly basic medium, low yield of the *para*-bromo-substituted derivative **2e** was observed presumably because of solubility reasons. Furthermore, the reaction of **1** with aldehydes containing unprotected OH group(s) did not result in the formation of the desired chalcones at all, except when methoxymethyl-ether (MOM) protected reagents were employed. However, in order to avoid the use of carcinogenic protecting groups, and to produce chalcones in a uniform manner, a pyrrolidine-catalyzed pathway was finally used to perform all the scale-up syntheses.

The condensation reactions were carried out in DMSO at 60 °C for 3 h in the presence of a catalytic amount of pyrrolidine (Table 1, entries 1–11) and almost complete conversions were achieved under these conditions independently of the reagents applied. However, beside the desired chalcones the formation of flavanone side-products *via* an *oxa*-Michael addition prevailed [33]. Chromatographic separation of the two compounds proved to be difficult, but at the same time the desired chalcones can be obtained simply by treating the mixture with methanolic KOH to induce ring opening of the flavanone moiety [34]. During the transformations, the 17-acetates were also deacetylated in all cases in the strongly basic medium. Compounds **2a**-**k** were thus obtained in moderate to good yields (55–86 %) after chromatographic purification. The presence of the aromatic or heteroaromatic ring as well as the α,β-unsaturated moiety were clearly confirmed by $^{1}H$ and $^{13}C$ NMR spectra of compounds **3**. Although the products are capable of geometric isomerism, the thermodynamically more stable (*E*)-isomers were formed exclusively presumably due to steric reasons.

As a continuation, the resulting chalcones were intended to be converted to flavones. Although a number of flavone syntheses are based on the base-catalyzed Baker-Venkataraman rearrangement of 2-acyloxyacetophenones to β-diketones and subsequent acid-induced cyclodehydration [27,28,35], these methods suffer from several drawbacks, such as the need for strong bases and acids, long reaction times or low yields of the desired products [36]. Some publications describe the applicability of $I_2$/DMSO system for the oxidative cyclization accompanied by *O*-deallylation of 2-allyloxy-chalcones and 2-allyloxy-α,β-dibromo-chalcones to flavones [37–39], while others report on the ICl/DMSO-catalyzed synthesis of flavones from 2-hydroxychalcones [40]. In all of these cases, at least an equivalent amount of reagents was used, although iodination of the flavone at position C-3 also occurred by applying the iodine in excess or prolonging the reaction time [39]. However, iodine was also found to be effective in a catalytic amount for the oxidative cyclization of chalcones derived from acetophenones with salicylaldehyde under heating in solvent-free conditions [41].

Based on these previous results, compound **2c** was dissolved in DMSO and 0.05 equiv. of iodine was added. After heating the mixture at 130 °C for 1 h, complete conversion was observed. According to NMR and ESI-MS measurements of the purified product, the corresponding flavone **3c** was obtained in excellent yield (91 %, Table 1, entry 3). Consequently, the same transformation was also carried out for the other chalcones (**2d**-**k**) and heterochalcone analogs (**2a** and **2b**) in order to access estradiol-(hetero)flavone hybrids. Although complete conversion was not indicated by TLC monitoring in every case, prolonged reaction time (usually more than an hour) led to the formation of unidentified side products that made separation rather difficult. Nevertheless, the desired products (**3a**, **3b** and **3d**-**k**) were obtained in good yields (Table 1, entries 1, 2 and 4–11). The plausible mechanism for the synthesis of flavones (**3**) via the oxidative cyclization of chalcones (**2**) using $I_2$/DMSO is indicated in Fig. 2. The first step may involve the iodination of the phenolic substrate **2** followed by oxidative cyclization. The acidic environment, the higher temperature and the oxidative atmosphere of the reaction medium might assist the regeneration of iodine catalyst from the reaction of DMSO with HI [42].

The $^{1}H$ and $^{13}C$ NMR spectra recorded for the obtained derivatives (**3a**-**k**) clearly indicated the peaks characteristic for the 4*H*-pyran-4-one ring, such as the singlet of 3′-H at around 7.0 ppm as well as the 4′-carbonyl signal of about 178 ppm. The C-2′ carbon peak can be observed at around 163 ppm for the flavones (**3c**-**k**), and at around 146 ppm for the heteroaryl-substituted analogs (**3a** and **3b**).

In order to expand the compound library accessible for primary cytotoxicity screen, attempts to prepare some 2-pyrazolyl estradiol derivatives were also made as similar derivatives have previously been

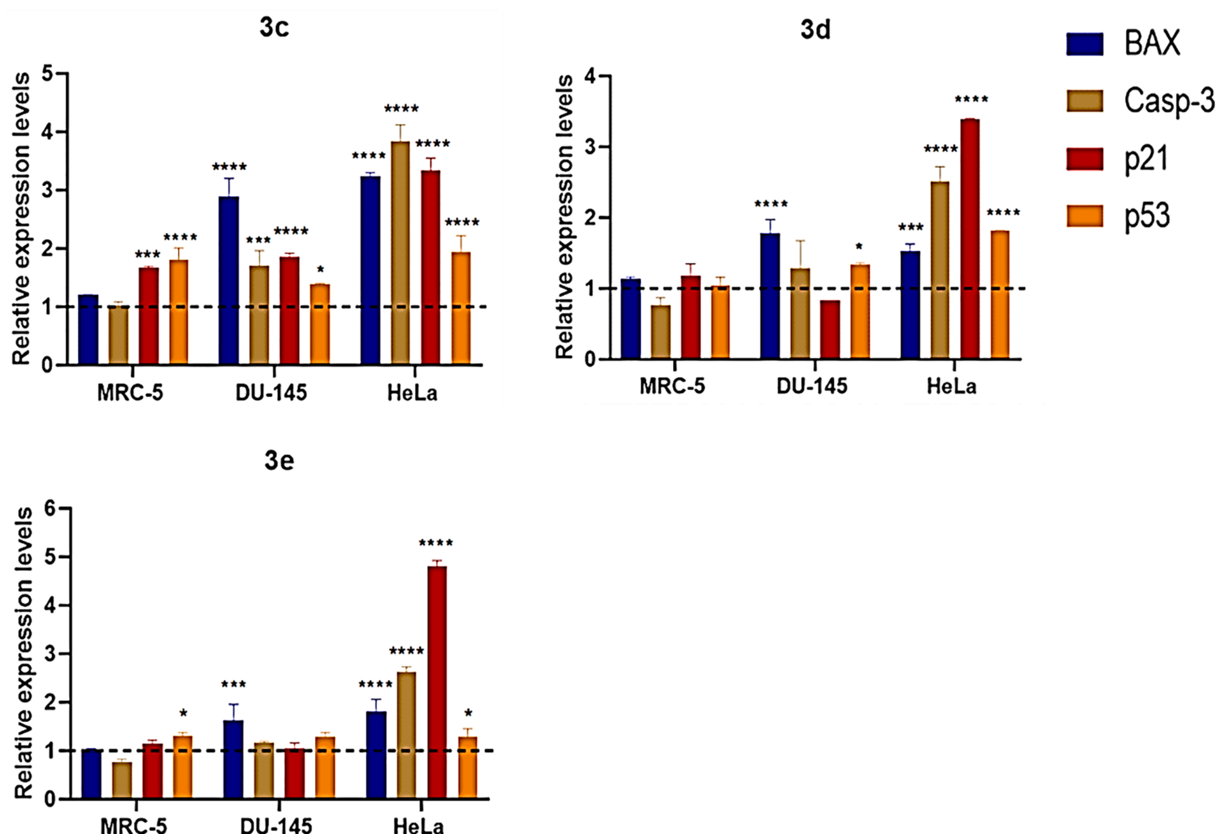



**Fig. 5.** Bar graphs representing relative mRNA levels of pro-apoptotic markers *BAX*, *Casp-3*, *p21*, *p53* in MRC-5, DU-145 and HeLa cells treated with test compounds **3c** (DU-145/HeLa: 3.2 μM, MRC-5: 4 μM), **3d** (DU-145: 6.9 μM, HeLa: 5.7 μM, MRC-5: 7.6 μM) and **3e** (DU-145: 4 μM, HeLa: 5.5 μM, MRC-5: 3 μM). The values represent the mean ± standard deviation calculated from three independent experiments (*P < 0.03, ***P < 0.0002, ****P < 0.0001, Fisher's LSD test).

found to be effective anticancer agents [29]. Although the α,β-unsaturated carbonyl moiety of chalcones, such in **3a**-**k**, would also be suitable for pyrazole formation with hydrazine or its derivatives, such reactions usually necessitate two reaction steps, *i.e.* cyclization to chiral pyrazoline and subsequent oxidation to aromatic pyrazole [27]. In contrast, opening the heterocyclic ring of flavones with hydrazine is also possible to give 2-pyrazolophenols in a single step, although these reactions generally require boiling of the reactants in EtOH for a long time (8–12 h) [43,44]. However, all of our early attempts at converting flavone **3c** into the corresponding pyrazol have failed. Utilizing conventional heating techniques, no product formation was observed even in the presence of large excesses of hydrazine hydrate. Prolonged refluxing (up to 72 h) also failed to yield the desired compound. In order to increase the reaction temperature and to achieve rate acceleration, microwave (MW) irradiation was next used in a closed vessel at 120 °C to carry out the reaction of **3c** with hydrazine hydrate (Scheme 1). To our delight, product formation was clearly visible by TLC monitoring. However, a large excess of hydrazine hydrate and long irradiation time (2 h) were still required for sufficient conversion of **3c**, although the corresponding product (**4c**) was obtained in good yield (78 %) after purification.

According to the $^{1}$H NMR spectrum of **4c**, the 2-pyrazolophenol moiety exists as a mixture of OH-N and NH-O tautomers. The separate signals observed for the nuclei belonging to and near the pyrazole ring indicated that the equilibration is slow at the NMR time scale (Fig. 3). The presence of the aforementioned tautomers was also evidenced by the $^{13}$C NMR spectrum, which clearly showed the individual peaks of the major and minor isomers. Recording the spectrum of **4c** after letting it stand in solution for a few days resulted in the coalescence of the $^{1}$H peaks in the aromatic region as well (Fig. 3). This anomalous behavior made it somewhat difficult to properly characterize this compound.

Due to the necessity of long reaction time for the MW-induced synthesis, the large excess of reagent needed, the anomalous behavior of the compounds in NMR and because of the complete pharmacological inefficacy of **4c** in the preliminary tests performed, the preparation of additional pyrazole derivatives was abandoned.

### 3.2. *Pharmacology*

With two different sets of compounds, namely estradiol-chalcones (**2a**–**k**) and flavones (**3a**-**k**) in our hands, our next goal was to investigate which structures may be of pharmacological interest as anticancer agents. Therefore, all derivatives were subjected to a preliminary *in vitro* cytotoxicity screen on MCF-7 breast, and HeLa cervical cancer, two prostate adenocarcinoma (DU-145, PC-3) cell lines as well as on non-cancerous fibroblasts (MRC-5) using MTT assays [45]. Parallel to the hybrid compounds the toxicity of estradiol and apigenin and of compound **4c** was assessed.

To perform the pharmacological screening, all A-ring-integrated estradiol-chalcones (**2a**–**k**) and -flavones (**3a**–**k**), the 2-pyrazolyl-estradiol derivative **4c**, as well as estradiol and apigenin were solubilized in DMSO at a final 10 mM concentration. The various human cancerous cell lines and MRC-5 fibroblasts were treated with the test compounds in 2.5 μM concentration for 72 h, then MTT assay was carried out. The acquired cell viability results are represented as a heat map (Fig. 4). We found that none of the estradiol-chalcone hybrids (**2a**–**k**) nor the 2-pyrazolyl-estradiol derivative **4c** showed any mentionable anticancer potential, they were practically ineffective on human cells. However, some of the flavone-containing compounds (**3c**, **3d**, **3e**) exhibited prominent cytotoxicity and proved to be highly efficient on all the tested cancer cells but not on non-cancerous fibroblasts. Another flavone hybrid, namely **3i** exerted strong cytotoxic effects on HeLa and PC-3 cells,

nevertheless it was just as detrimental to non-cancerous MRC-5 cells as well. Apigenin (Api) as well as estradiol (E2) were non-toxic to human cells at 2.5 μM concentration (Fig. 4). Therefore, based on these results, compounds **3c**, **3d** and **3e** were identified as positive hits, showing distinguished and selective anticancer performance. Following the primary screen, compound **3c**, **3d** and **3e** (72 h, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, 7.2 8.0 μM) and a reference chemotherapy drug cisplatin (24 h, 20, 40, 60, 80, 330 μM) were applied on all five cell lines. After treatments the $IC_{50}$ values of the three selected compounds and cisplatin were determined (Table 2). Based on the results it can be concluded that the $IC_{50}$ values of **3c**, **3d** and **3e** are notably lower than those of cisplatin in the case of all five cell lines. Moreover, the $IC_{50}$ values of the steroidal compounds on non-cancerous fibroblasts (MRC-5) are usually higher than the values obtained on cancerous cells, contrary to cisplatin which proved to be overall more toxic to fibroblasts than to cancer cells. Some of the $IC_{50}$ values of **3d** and **3e**, but especially of **3c** obtained on HeLa, MCF-7 and DU-145 cells were notably lower compared to that on MRC-5 non-cancerous fibroblasts, despite falling in the same order of magnitude.

Evading programmed cell death, specifically apoptosis, is one of the key characteristics of most, if not all types of cancer cells [46]. Therefore, inducing apoptosis in cancer cells is a viable therapeutic approach [47]. In fact, a handful of commercially available steroid derivatives exhibit their anticancer effect by triggering apoptosis [48]. Hence, the apoptosis inducing potential of estradiol-flavone hybrids **3c**, **3d** and **3e** was examined by assessing the expression of apoptotic genes at the mRNA level with the reverse transcription quantitative PCR method (RT-qPCR). Following treatments of cancerous DU-145 and HeLa and of non-cancerous MRC-5 cells with the appropriate molecules **3c**, **3d** and **3e**, mRNA was isolated, reverse transcribed and the relative mRNA levels of some pro-apoptotic genes (*BAX*, *Casp-3*, *p21*, *p53*) were measured by RT-qPCR.

Our results indicate that all three compounds were capable of upregulating the expression of pro-apoptotic genes in a statistically significant manner (Fig. 5). In line with the cytotoxicity results higher expression of pro-apoptotic markers was observed in estradiol-flavone hybrid-treated cancerous cell lines (DU-145, HeLa) compared to non-cancerous MRC-5 fibroblasts. Between the two cancer cell lines, HeLa cells were the most affected ones, as all pro-apoptotic genes showed transcriptional activation upon treatment with each test molecule. Compound **3c** induced most significantly the expression levels of all pro-apoptotic genes in both cancer cell lines, however, the apoptosis triggering effect of compound **3d** showed the highest cancer cell selectivity.

## 4. Conclusions

In conclusion, several novel estradiol-chalcone hybrids were synthesized from 2-acetyl-estradiol-17β-acetate without the use of hydroxyl protecting groups. The oxidative cyclizations performed with a catalytic amount of iodine in DMSO furnished the corresponding estradiol-flavone hybrids, and the possibility of further transformations into pyrazole derivatives was briefly explored. The synthesized compounds have been tested for *in vitro* cytotoxic activity on several cancerous cell lines. These data revealed a structure–activity relationship with estradiol-flavone hybrids exerting cancer cell-selective toxicity. Surprisingly, derivatives structurally related to naturally occurring HO- and MeO-substituted flavones and all the estradiol-chalcone compounds were found ineffective, at least in the cell lines tested. The $IC_{50}$ concentrations of the most promising steroids were found to be significantly lower than those of cisplatin in the tested cell lines. The apoptosis inducing activity of the most efficient cytotoxic flavone hybrids was verified implying a drug-induced apoptotic elimination of cancer cells.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

The list, sequence and appropriate concentrations of primers used for qRT-PCR (Table S1). Dose-response curves used to determine the $IC_{50}$ concentrations of selected compounds (Fig. S1). $^{1}H$ and $^{13}C$ NMR spectra of the synthesized compounds. Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2022.109099.

## References

[1] C. Viegas-Junior, A. Danuello, V. da S. Bolzani, E. J. Barreiro, C. A. M. Fraga, Molecular hybridization: a useful tool in the design of new drug prototypes, Curr. Med. Chem. 14 (2007) 1829–1852. 10.2174/092986707781058805.

[2] V. Ivasiv, C. Albertini, A.E. Gonçalves, M. Rossi, M.L. Bolognesi, Molecular hybridization as a tool for designing multitarget drug candidates for complex diseases, Curr. Top. Med. Chem. 19 (2019) 1694–1711, https://doi.org/10.2174/1568026619666190619115735.

[3] G. Mehta, V. Singh, Hybrid systems through natural product leads: an approach towards new molecular entities, Chem. Soc. Rev. 31 (2002) 324–334, https://doi.org/10.1039/B204748A.

[4] G. Bérubé, An overview of molecular hybrids in drug discovery, Expert Opin. Drug Discov. 11 (2016) 281–305, https://doi.org/10.1517/17460441.2016.1135125.

[5] M. Chandrashekhar, V.L. Nayak, S. Ramakrishna, U.V. Mallavadhani, Novel triazole hybrids of myrrhanone C, a natural polypodane triterpene: Synthesis, cytotoxic activity and cell based studies, Eur. J. Med. Chem. 114 (2016) 293–307, https://doi.org/10.1016/j.ejmech.2016.03.013.

[6] C. Madasu, S. Karri, R. Sangaraju, R. Sistla, M.V. Uppuluri, Synthesis and biological evaluation of some novel 1,2,3-triazole hybrids of myrrhanone B isolated from Commiphora mukul gum resin: identification of potent antiproliferative leads active against prostate cancer cells (PC-3), Eur. J. Med. Chem. 188 (2020), 111974, https://doi.org/10.1016/j.ejmech.2019.111974.

[7] C. Madasu, Y.-M. Xu, E.M.K. Wijeratne, M.X. Liu, I. Molnár, A.A.L. Gunatilaka, Semi-synthesis and cytotoxicity evaluation of pyrimidine, thiazole, and indole analogues of argentatins A-C from guayule (Parthenium argentatum) resin, Med. Chem. Res. 31 (2022) 1088–1098, https://doi.org/10.1007/s00044-021-02835-1.

[8] P. Borah, P. Chowdhury, Steroid hybrid systems: new molecular entities with potential therapeutical spectrum, Curr. Drug Ther. 12 (2017) 3–22, https://doi.org/10.2174/1574885512666170109143715.

[9] L. Almagro, D. Hernández-Castillo, O. Ortiz, D. Alonso, A. Ruiz, J. Coro, M.Á. Herranz, D. Molero, R. Martínez-Álvarez, M. Suárez, N. Martín, Steroid–fullerene hybrids from epiandrosterone: synthesis, characterization and theoretical study, Eur. J. Org. Chem. 2018 (2018) 4512–4522, https://doi.org/10.1002/ejoc.201800622.

[10] Á. Baji, A. Gyovai, J. Wölfling, R. Minorics, I. Ocsovszki, I. Zupkó, É. Frank, Microwave-assisted one-pot synthesis of steroid–quinoline hybrids and an evaluation of their antiproliferative activities on gynecological cancer cell lines, RSC Adv. 6 (2016) 27501–27516, https://doi.org/10.1039/C6RA03910C.

[11] J. Coro, H. Rodríguez, D.G. Rivera, M. Suárez, D. Molero, M.Á. Herranz, R. Martínez-Álvarez, S. Filippone, N. Martín, Functional chimeras: new Bingel–Hirsch-type steroid–fullerene hybrids, Eur. J. Org. Chem. 2009 (2009) 4810–4817, https://doi.org/10.1002/ejoc.200900583.

[12] L.F. Tietze, G. Schneider, J. Wölfling, A. Fecher, T. Nöbel, S. Petersen, I. Schuberth, C. Wulff, A novel approach in drug discovery: synthesis of estrone–talaromycin

natural product hybrids, Chem. – Eur. J. 6 (2000) 3755–3760, https://doi.org/10.1002/1521-3765(20001016)6:20<3755::AID-CHEM3755>3.0.CO;2-L.
[13] G. Shagufta, Panda, A new example of a steroid–amino acid hybrid: construction of constrained nine membered D-ring steroids, Org. Biomol. Chem. 5 (2007) 360–366, https://doi.org/10.1039/B616155C.
[14] Q. Hou, X. Lin, X. Lu, C. Bai, H. Wei, G. Luo, H. Xiang, Discovery of novel steroidal-chalcone hybrids with potent and selective activity against triple-negative breast cancer, Bioorg. Med. Chem. 28 (2020) 115763. doi: 10.1016/j.bmc.2020.115763.
[15] M. Monier, A. El-Mekabaty, D. Abdel-Latif, B.D. Mert, K.M. Elattar, Heterocyclic steroids: efficient routes for annulation of pentacyclic steroidal pyrimidines, Steroids 154 (2020), 108548, https://doi.org/10.1016/j.steroids.2019.108548.
[16] A. Gupta, B.S. Kumar, A.S. Negi, Current status on development of steroids as anticancer agents, J. Steroid Biochem. Mol. Biol. 137 (2013) 242–270, https://doi.org/10.1016/j.jsbmb.2013.05.011.
[17] G.M. Anstead, K.E. Carlson, J.A. Katzenellenbogen, The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site, Steroids. 62 (1997) 268–303, https://doi.org/10.1016/S0039-128X(96)00242-5.
[18] É. Frank, G. Schneider, Synthesis of sex hormone-derived modified steroids possessing antiproliferative activity, J. Steroid Biochem. Mol. Biol. 137 (2013) 301–315, https://doi.org/10.1016/j.jsbmb.2013.02.018.
[19] N.J. Lakhani, M.A. Sarkar, J. Venitz, W.D. Figg, 2-Methoxyestradiol, a promising anticancer agent, Pharmacother. J. Hum. Pharmacol, Drug Ther. 23 (2003) 165–172, https://doi.org/10.1592/phco.23.2.165.32088.
[20] S. Verenich, P.M. Gerk, Therapeutic promises of 2-methoxyestradiol and its drug disposition challenges, Mol. Pharm. 7 (2010) 2030–2039, https://doi.org/10.1021/mp100190f.
[21] B. Molnár, N.I. Kinyua, G. Mótyán, P. Leits, I. Zupkó, R. Minorics, G.T. Balogh, É. Frank, Regioselective synthesis, physicochemical properties and anticancer activity of 2-aminomethylated estrone derivatives, J. Steroid Biochem. Mol. Biol. 219 (2022), 106064, https://doi.org/10.1016/j.jsbmb.2022.106064.
[22] G. Panchapakesan, V. Dhayalan, N.D. Moorthy, N. Saranya, A.K. Mohanakrishnan, Synthesis of 2-substituted 17β-hydroxy/17-methylene estratrienes and their in vitro cytotoxicity in human cancer cell cultures, Steroids 76 (2011) 1491–1504, https://doi.org/10.1016/j.steroids.2011.08.004.
[23] C. Bubert, M.P. Leese, M.F. Mahon, E. Ferrandis, S. Regis-Lydi, P.G. Kasprzyk, S. P. Newman, Y.T. Ho, A. Purohit, M.J. Reed, B.V.L. Potter, 3,17-Disubstituted 2-alkylestra-1,3,5(10)-trien-3-ol derivatives: synthesis, in vitro and in vivo anticancer activity, J. Med. Chem. 50 (2007) 4431–4443, https://doi.org/10.1021/jm070405v.
[24] A.B. Edsall, A.K. Mohanakrishnan, D. Yang, P.E. Fanwick, E. Hamel, A.D. Hanson, G.E. Agoston, M. Cushman, Effects of altering the electronics of 2-methoxyestradiol on cell proliferation, on cytotoxicity in human cancer cell cultures, and on tubulin polymerization, J. Med. Chem. 47 (2004) 5126–5139, https://doi.org/10.1021/jm049647a.
[25] C. Wang, L. Li, D. Fu, T. Qin, Y. Ran, F. Xu, X. Du, H. Gao, S. Sun, T. Yang, X. Zhang, J. Huo, W. Zhao, Z. Zhang, X. Shi, Discovery of chalcone-modified estradiol analogs as antitumour agents that Inhibit tumour angiogenesis and epithelial to mesenchymal transition, Eur. J. Med. Chem. 176 (2019) 135–148, https://doi.org/10.1016/j.ejmech.2019.04.071.
[26] R. Dutour, F. Cortés-Benítez, J. Roy, D. Poirier, Structure-based design and synthesis of new estrane–pyridine derivatives as cytochrome P450 (CYP) 1B1 inhibitors, ACS Med. Chem. Lett. 8 (2017) 1159–1164, https://doi.org/10.1021/acsmedchemlett.7b00265.
[27] A. Omar, I. Ahmed, O. AboulWafa, A. Hassan, K. Ismail, M. El-Din, N. Mansour, Synthesis, binding affinities and uterotrophic activity of some 2-substituted estradiol and ring-A-fused pyrone derivatives, Eur. J. Med. Chem. 29 (1994) 25–32, https://doi.org/10.1016/0223-5234(94)90122-8.
[28] F. Kovács, M.K. Gopisetty, D.I. Adamecz, M. Kiricsi, É.A. Enyedy, É. Frank, Synthesis and conversion of primary and secondary 2-aminoestradiols into A-ring-integrated benzoxazolone hybrids and their in vitro anticancer activity, RSC Adv. 11 (2021) 13885–13896, https://doi.org/10.1039/D1RA01889B.
[29] B. Molnár, M.K. Gopisetty, D.I. Adamecz, M. Kiricsi, É. Frank, Multistep synthesis and in vitro anticancer evaluation of 2-pyrazolyl-estradiol derivatives, pyrazolocoumarin-estradiol hybrids and analogous compounds, Molecules. 25 (2020) 4039, https://doi.org/10.3390/molecules25184039.
[30] Z. Rozmer, P. Perjési, Naturally occurring chalcones and their biological activities, Phytochem. Rev. 15 (2016) 87–120, https://doi.org/10.1007/s11101-014-9387-8.
[31] X. Yan, M. Qi, P. Li, Y. Zhan, H. Shao, Apigenin in cancer therapy: anti-cancer effects and mechanisms of action, Cell Biosci. 7 (2017) 50, https://doi.org/10.1186/s13578-017-0179-x.
[32] K. Nepali, S. Sharma, M. Sharma, P.M.S. Bedi, K.L. Dhar, Rational approaches, design strategies, structure activity relationship and mechanistic insights for anticancer hybrids, Eur. J. Med. Chem. 77 (2014) 422–487, https://doi.org/10.1016/j.ejmech.2014.03.018.
[33] M.M. Naik, S.G. Tilve, V.P. Kamat, Pyrrolidine and iodine catalyzed domino aldol-Michael-dehydrogenative synthesis of flavones, Tetrahedron Lett. 55 (2014) 3340–3343, https://doi.org/10.1016/j.tetlet.2014.04.051.
[34] A. Cisak, C. Mielczarek, Practical and theoretical aspects of flavanone–chalcone isomerisations, J. Chem. Soc. Perkin Trans. 2 (1992) 1603–1607, https://doi.org/10.1039/P29920001603.
[35] C. M. M. Santos, A. M. S. Silva, Chapter 6.4 – Six-membered ring systems: with O and/or S atoms, in: Gribble, G. W. and Joule, J. A. (Eds.), Prog. Heterocycl. Chem., Elsevier, 2015: pp. 465–529. 10.1016/B978-0-08-100024-3.00015-5.
[36] D. Ameen, T.J. Snape, Mechanism and application of Baker-Venkataraman O→C acyl migration reactions, Synthesis. 47 (2015) 141–158, https://doi.org/10.1055/s-0034-1379498.
[37] P.D. Lokhande, S.S. Sakate, K.N. Taksande, B. Navghare, Dimethylsulfoxide–iodine catalysed deprotection of 2′-allyloxychalcones: synthesis of flavones, Tetrahedron Lett. 46 (2005) 1573–1574, https://doi.org/10.1016/j.tetlet.2004.12.041.
[38] B.R. Nawghare, S.V. Gaikwad, A. Raheem, P.D. Lokhande, Iodine catalyzed cascade synthesis of flavone derivatives from 2'-allyloxy-α, β-dibromochalcones, J. Chil. Chem. Soc. 59 (2014) 2284–2286, https://doi.org/10.4067/S0717-97072014000100009.
[39] A.M. Patil, D.A. Kamble, P.D. Lokhande, Iodine-mediated direct synthesis of 3-iodoflavones, Synth. Commun. 48 (2018) 1299–1307, https://doi.org/10.1080/00397911.2018.1440601.
[40] A. Lahyani, M. Trabelsi, Ultrasonic-assisted synthesis of flavones by oxidative cyclization of 2′-hydroxychalcones using iodine monochloride, Ultrason. Sonochem. 31 (2016) 626–630, https://doi.org/10.1016/j.ultsonch.2016.02.018.
[41] K.V. Sashidhara, M. Kumar, A. Kumar, A novel route to synthesis of flavones from salicylaldehyde and acetophenone derivatives, Tetrahedron Lett. 53 (2012) 2355–2359, https://doi.org/10.1016/j.tetlet.2012.02.108.
[42] A. Monga, S. Bagchi, A. Sharma, Iodine/DMSO oxidations: a contemporary paradigm in C-N bond chemistry, New J. Chem. 42 (2018) 1551–1576, https://doi.org/10.1039/C7NJ04513A.
[43] B.P. Bandgar, J.V. Totre, S.S. Gawande, C.N. Khobragade, S.C. Warangkar, P. D. Kadam, Synthesis of novel 3,5-diaryl pyrazole derivatives using combinatorial chemistry as inhibitors of tyrosinase as well as potent anticancer, anti-inflammatory agents, Bioorg. Med. Chem. 18 (2010) 6149–6155, https://doi.org/10.1016/j.bmc.2010.06.046.
[44] C.-Y. Cui, J. Liu, H.-B. Zheng, X.-Y. Jin, X.-Y. Zhao, W.-Q. Chang, B. Sun, H.-X. Lou, Diversity-oriented synthesis of pyrazoles derivatives from flavones and isoflavones leads to the discovery of promising reversal agents of fluconazole resistance in Candida albicans, Bioorg. Med. Chem. Lett. 28 (2018) 1545–1549, https://doi.org/10.1016/j.bmcl.2018.03.066.
[45] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63, https://doi.org/10.1016/0022-1759(83)90303-4.
[46] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70, https://doi.org/10.1016/S0092-8674(00)81683-9.
[47] C.M. Pfeffer, A.T.K. Singh, Apoptosis: a target for anticancer therapy, Int. J. Mol. Sci. 19 (2018) 448, https://doi.org/10.3390/ijms19020448.
[48] R. Gerl, D.L. Vaux, Apoptosis in the development and treatment of cancer, Carcinogenesis 26 (2005) 263–270, https://doi.org/10.1093/carcin/bgh283.