

# Pd-Catalyzed Suzuki–Miyaura couplings and evaluation of 13 $\alpha$ -estrone derivatives as potential anticancer agents

Rebeka Jójárt,<sup>1,a</sup> Hazhmat Ali,<sup>2,a</sup> Gergely Horváth,<sup>1</sup> Zoltán Kele,<sup>3</sup> István Zupkó,<sup>2\*</sup> Erzsébet Mernyák<sup>1\*</sup>

<sup>1</sup>*Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary*

<sup>2</sup>*Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6., H-6720 Szeged, Hungary*

<sup>3</sup>*Department of Medicinal Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary*

\*Corresponding authors. Tel.: +36 62 544277; fax: +36 62 544200 (E. Mernyák).

E-mail addresses: bobe@chem.u-szeged.hu (E. Mernyák).

<sup>a</sup>These authors contributed equally to this work.

## Abstract

13 $\alpha$ -Estrones are of great value owing to their potent multiple bioactivity, including anticancer activity. 3-OH or 3-OBn derivatives of 2- or 4-[(subst.) phenyl]-13 $\alpha$ -estrone as potential antiproliferative agents have been synthesized via facile, microwave-induced, Pd-catalyzed Suzuki–Miyaura coupling. 2- or 4-Halogenated 13 $\alpha$ -estrone derivatives have been reacted with (4-subst.)phenylboronic acids using Pd(PPh<sub>3</sub>)<sub>4</sub> as catalyst. The nature of *para* substituents at the introduced phenyl group did not influence the outcome of couplings. Certain newly synthesized compounds displayed substantial antiproliferative action against human adherent cancer cell lines of gynecological origin. Important structure–activity relationships were revealed, which might be helpful in the design of potent and selective anticancer derivatives based on the hormonally inactive 13 $\alpha$ -estrane core.

**Keywords:** Suzuki–Miyaura coupling, 13 $\alpha$ -estrone, phenylboronic acid, anticancer, MCF-7, Hela.

## 1. Introduction

The primary function of female sex hormones (estrogens) is to facilitate the development of female secondary sexual characteristics and to control the reproductive functions [1]. However, certain estrogens possess other biological activities, including anti-angiogenic, neuroprotective, anticancer effects and others [2]. Despite the known property of 17 $\beta$ -estradiol promoting cell

proliferation, its derivatives might act as antiproliferative agents [3]. The major risk in the design of anticancer agents based on the estrane core is the potential hormonal side effect of the lead compound. One possibility for minimizing the undesirable estrogenic behavior is the transformation of natural estrogens into their core-modified analogs [4–6]. 13 $\alpha$ -Estrone (**1a**) is an epimer of natural estrone (Figure 1) [4]. The inversion of the configuration at C-13 results in a modified conformation, and that is why 13 $\alpha$ -estrone and its 17-ol derivatives do not possess estrogenic behavior [7]. Considering that 13 $\alpha$ -estrone (**1a**) is readily available in a single step from its natural counterpart, it might serve as a promising candidate for the design of anticancer compounds lacking hormonal action. We have demonstrated recently that this group of synthetic compounds offers great possibilities concerning bioactivity [8–10]. It was shown that certain structurally different enzymes involved in estrogen biosynthesis [11,12] can be inhibited by 2- and/or 4-substituted 13 $\alpha$ -estrone derivatives (Figure 2, compound **I**). The most potent inhibitors displayed IC<sub>50</sub> values in ranges similar to those of their 13 $\beta$  counterparts. It should be highlighted that organic anion transporter protein OATP2B1, one of the key players in intestinal drug absorption and drug transport, can also be inhibited by 13 $\alpha$ -estrone compounds (Figure 2, compound **3a**) [13]. The observed inhibitory potencies are comparable to those of the best OATP2B1 inhibitors ever published.

Figure 1.

Not only enzyme inhibitory behavior, but the direct antiproliferative potential of certain 13 $\alpha$ -estrones have also been published by our group [14–20]. Certain C-3-*O* and C-16 modified derivatives (Figure 2, compound **II** [14]) displayed outstanding cell growth-inhibitory action against a range of human adherent cancer cell lines. Investigations of the mechanism of action revealed that compound **II** indicated cell cycle blockade at the G2–M transition. Caspase activity determinations proved that the compound induced apoptosis via the intrinsic pathway.

Figure 2.

The above-mentioned literature data suggest that 13 $\alpha$ -estrones are of great value owing to their potent multiple bioactivity without estrogenic side effects.

The Suzuki–Miyaura reaction is a palladium-catalyzed cross-coupling between organic boron compounds and organic halides [21,22]. It requires the addition of a base for activation of a boron reagent. The C(sp<sup>2</sup>)–C(sp<sup>2</sup>) coupling proceeds under mild conditions and tolerates a wide range of functional groups. This methodology is a very powerful tool for diaryl formation, including the synthesis of pharmaceuticals and complex natural products.

Literature reveals the application of Suzuki–Miyaura coupling in the synthesis of estrone derivatives with diverse biological activities. C–C couplings on the estrane core were carried out mainly at C-2, C-3, C-4 or C-17 [23–29]. Estrone derivatives such as aryl halides (at C-2 or C-4) or aryl or enol triflates (at C-3 or C-17) have been used. However, Ciana et al. developed a copper-catalyzed, site-selective C–H arylation methodology for the synthesis of 2-phenyl estrone derivatives [30]. Sato et al. synthesized estrone–isoquinoline hybrids as cortistatin analogs with substantial anti-angiogenic properties [23]. 17-Pyridyl estrones have been prepared as effective AKR1C3 inhibitors, which might be promising drug candidates in the treatment of endometriosis. Suzuki couplings at C-3 resulted in estrone derivatives as covalent 17 $\beta$ -HSD1 inhibitors [24]. Langer et al. published recently the synthesis of 2- and/or 4-arylestrones via C–C coupling. Certain 4-aryl regioisomers proved to be potent pancreatic lipase inhibitors [25]. Poirier et al. demonstrated that estrone derivatives bearing 2-(subst.)phenyl ring possess substantial cytochrome P450 (CYP) 1B1 inhibitory activity [29]. Based on the above-mentioned literature results, here we intended to perform Suzuki–Miyaura couplings on the hormonally inactive 13 $\alpha$ -estrane core. Regarding the promising biological properties of derivatives modified on ring A, C-2 and C-4 have been chosen as subjects for modifications. Our aim was to synthesize 3-hydroxy and 3-benzyloxy 2- or 4-(subst.)phenyl compounds. The determination of antiproliferative properties of the newly synthesized 13 $\alpha$ -estrone derivatives against a panel of five human adherent gynecological cancer cell lines (MCF-7, MDA-MB-231, Hela, SiHa, A2780) was also performed.

## 2. Experimental

### 2.1. Chemistry

Melting points (Mp) were determined with a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer CHN analyzer model 2400. Thin-layer chromatography: silica gel 60 F254; layer thickness 0.2 mm (Merck); eluents (ss): 30% ethyl acetate/70% hexane, detection with I<sub>2</sub> or UV (365 nm) after spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid and heating at 100–120 °C for 10 min. Flash chromatography: silica gel 60, 40–63  $\mu$ m (Merck). Reactions under microwave irradiation were

carried out with a CEM Corporation focused microwave system, Model Discover SP. The maximum power of irradiation was 200 W.  $^1\text{H}$  NMR spectra were recorded in  $\text{DMSO-d}_6$ ,  $\text{CDCl}_3$  solution with a Bruker DRX-500 instrument at 500 MHz, with  $\text{Me}_4\text{Si}$  as internal standard.  $^{13}\text{C}$  NMR spectra were recorded with the same instrument at 125 MHz under the same conditions. Mass spectrometry: full scan mass spectra of the compounds were acquired in the range of 50 to 1000  $m/z$  with a Finnigan TSQ-7000 triple quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA) equipped with a Finnigan electrospray ionization source. Analyses were performed in positive ion mode using flow injection mass spectrometry with a mobile phase of 50% aqueous acetonitrile containing 0.1 v/v% formic acid. The flow rate was 0.3 ml/min. Five  $\mu\text{l}$  aliquot of the samples were loaded into the flow. The ESI capillary was adjusted to 4.5 kV and  $\text{N}_2$  was used as a nebulizer gas.

#### 2.1.1. Reaction conditions for Suzuki-Miyaura coupling in 3-OH series

2-Bromo-3-hydroxy-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**3a**, 175 mg, 0.50 mmol) or 4-bromo-3-hydroxy-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**6a**, 175 mg, 0.50 mmol),  $\text{Pd}(\text{PPh}_3)_4$  (58 mg, 0.050 mmol, 0.1 equiv),  $\text{K}_2\text{CO}_3$  (276 mg, 2.0 mmol, 4 equiv.), (4-subst.)phenylboronic acid (2 equiv.) and toluene (4 ml) were added under nitrogen atmosphere, in 10 mL Pyrex pressure vessels (CEM, Part #: 908035) with silicone cap (CEM, Part #: 909210) and the mixture was heated in a CEM microwave reactor at 100  $^\circ\text{C}$  for 30 min under stirring. The solvent was evaporated in vacuo. The residue was purified by flash chromatography with 10% ethyl acetate/ 90% hexane as eluent.

##### 2.1.1.1. 3-Hydroxy-2-phenyl-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**11a**)

Compound **11a** was isolated as white crystals (165 mg, 95%). Mp.: 197–205  $^\circ\text{C}$ .  $R_f$  = 0.46. Anal calcd. for  $\text{C}_{24}\text{H}_{26}\text{O}_2$ : C, 83.20; H, 7.56. Found: 83.28; H, 7.61.  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  ppm: 0.96 (s, 3H, H-18), 2.72 (m, 2H, H-6), 6.59 (s, 1H, H-4), 7.08 (s, 1H, H-1), 7.25 (t,  $J=7.5$  Hz, 1H, H-4'), 7.34 (t,  $J=7.5$  Hz, 2H, H-3' and H-5'), 7.49 (d,  $J=7.1$  Hz, 2H, H-2' and H-6'), 9.17 (s, 1H, 3-OH).  $^{13}\text{C}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  ppm: 20.4 ( $\text{CH}_2$ ), 24.5 (C-18), 27.7 ( $\text{CH}_2$ ), 28.0 ( $\text{CH}_2$ ), 29.2 ( $\text{CH}_2$ ), 31.6 ( $\text{CH}_2$ ), 32.8 ( $\text{CH}_2$ ), 40.6 (CH), 40.8 (CH), 48.5 (CH), 49.4 (C-13), 115.4 (C-4), 125.2 (C-2), 126.1 (C-4'), 127.6 (C-1), 127.7 (2C, 2 $\times$ CH), 128.9 (2C, 2 $\times$ CH), 130.3 (C), 136.8 (C), 138.8 (C), 151.8 (C-3), 220.6 (C-17). MSI  $m/z$  (%): 345 (100,  $[\text{M-H}]^-$ ).

##### 2.1.1.2. 3-Hydroxy-2-(4-chlorophenyl)-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**12a**)

Compound **12a** was isolated as white crystals (180 mg, 94%). Mp.: 256–257 °C.  $R_f$ =0.43. Anal calcd. for  $C_{24}H_{25}ClO_2$ : C, 75.68; H, 6.62. Found: 75.75; H, 6.53.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 0.97 (s, 3H, H-18), 2.72 (m, 2H, H-6), 6.60 (s, 1H, H-4), 7.10 (s, 1H, H-1), 7.40 (d,  $J$ =8.6 Hz, 2H, H-3' and H-5'), 7.52 (d,  $J$ =8.6 Hz, 2H, H-2' and H-6'), 9.31 (s, 1H, 3-OH).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 20.4 ( $CH_2$ ), 24.5 (C-18), 27.7 ( $CH_2$ ), 28.0 ( $CH_2$ ), 29.2 ( $CH_2$ ), 31.5 ( $CH_2$ ), 32.8 ( $CH_2$ ), 40.6 (CH), 40.8 (CH), 48.5 (CH), 49.4 (C-13), 115.5 (C-4), 123.8 (C-2), 127.4 (C-1), 127.7 (2C, 2 $\times$ CH), 130.5 (C), 130.6 (2C, 2 $\times$ CH), 130.7 (C), 137.3(C), 137.6 (C), 151.8 (C-3), 220.6 (C-17). MSI  $m/z$  (%): 379 (100,  $[M-H]^+$ ).

#### 2.1.1.3. 2-(4-*tert*-Butylphenyl)-3-hydroxy-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**13a**)

Compound **13a** was isolated as white crystals (187 mg, 93%). Mp.: 238–240 °C.  $R_f$ =0.54. Anal calcd. for  $C_{28}H_{34}O_2$ : C, 83.54; H, 8.51. Found: 83.61; H, 8.42.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 0.96 (s, 3H, H-18), 1.29 (s, 9H, 4'-C( $CH_3$ ) $_3$ ), 2.71 (m, 2H, H-6), 6.58 (s, 1H, H-4), 7.07 (s, 1H, H-1), 7.25 (t,  $J$ =7.5 Hz, 1H, H-4'), 7.40 (2xd,  $J$ =8.5 Hz, 2 $\times$ 2H, H-2', H-3', H-5' and H-6'), 9.14 (s, 1H, 3-OH).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 20.4 ( $CH_2$ ), 24.5 (C-18), 27.7 ( $CH_2$ ), 28.0 ( $CH_2$ ), 29.2 ( $CH_2$ ), 31.0 (3C, 4'-C( $CH_3$ ) $_3$ ), 31.6 ( $CH_2$ ), 32.8 ( $CH_2$ ), 34.0 (4'-C( $CH_3$ ) $_3$ ), 40.6 (CH), 40.8 (CH), 48.5 (CH), 49.4 (C-13), 115.4 (C-4), 124.5 (2C, 2 $\times$ CH), 125.1 (C-2), 127.6 (C-1), 128.6 (2C, 2 $\times$ CH), 130.3 (C), 135.9 (C), 136.5 (C), 148.3 (C), 151.8 (C-3), 220.6 (C-17). MSI  $m/z$  (%): 401 (100,  $[M-H]^+$ ).

#### 2.1.1.4. 3-Hydroxy-4-phenyl-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**14a**)

Compound **14a** was isolated as white crystals (163 mg, 94%). Mp.: 125–129 °C.  $R_f$ =0.56. Anal calcd. for  $C_{24}H_{26}O_2$ : C, 83.20; H, 7.56. Found: 83.29; H, 7.50.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 0.96 (s, 3H, H-18), 6.71 (d,  $J$ = 8.5 Hz, 1H, H-2), 7.08 (d,  $J$ = 8.5 Hz, 1H, H-1), 7.13 (t,  $J$ =7.4 Hz, 2H, H-3' and H-5'), 7.27 (t,  $J$ =7.4 Hz, 1H, H-4'), 7.36 (overlapping multiplets, 2H, H-2' and H-6'), 8.82 (s, 1H, 3-OH).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 20.9 ( $CH_2$ ), 24.9 (C-18), 28.3 ( $CH_2$ ), 28.7 ( $CH_2$ ), 29.3 ( $CH_2$ ), 32.2 ( $CH_2$ ), 33.3 ( $CH_2$ ), 40.8 (CH), 41.6 (CH), 49.0 (CH), 49.9 (C-13), 112.8 (C-2), 125.4 (CH), 126.2 (CH), 127.6(C), 127.8 (2C, 2 $\times$ CH), 129.9 (2C, 2 $\times$ CH), 130.2 (C), 135.5 (C), 137.9 (C), 152.0 (C-3), 220.6 (C-17). MSI  $m/z$  (%): 345 (100,  $[M-H]^+$ ).

#### 2.1.1.5. 3-Hydroxy-4-(4-chlorophenyl)-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**15a**)

Compound **12a** was isolated as white crystals (175 mg, 92%). Mp.: 71–78 °C.  $R_f=0.59$ . Anal calcd. for  $C_{24}H_{25}ClO_2$ : C, 75.68; H, 6.62. Found: 75.74; H, 6.56.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 0.96 (s, 3H, H-18), 6.71 (d,  $J=8.5$  Hz, 1H, H-2), 7.08 (d,  $J=8.5$  Hz, 1H, H-1), 7.10 (d,  $J=8.5$  Hz, 1H, H-1), 7.13 (m, 2H, H-3' and H-5'), 7.41 (m, 2H, H-2' and H-6'), 8.94 (s, 1H, 3-OH).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 20.3 (CH<sub>2</sub>), 24.4 (C-18), 27.7 (CH<sub>2</sub>), 28.2 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 32.8 (CH<sub>2</sub>), 40.2 (CH), 41.0 (CH), 48.4 (CH), 49.3 (C-13), 112.8 (C-2), 125.8 (CH), 126.3 (C), 127.8 (2C, 2 $\times$ CH), 130.2 (C), 131.0 (C), 131.8 (CH), 131.9 (CH), 135.4 (C), 136.7 (C), 152.0 (C-3), 220.6 (C-17). MSI  $m/z$  (%): 381 (100, [M+H]<sup>+</sup>).

#### 2.1.1.6. 4-(4-*tert*-Butylphenyl)-3-hydroxy-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**16a**)

Compound **13a** was isolated as white crystals (183 mg, 91%). Mp.: 211–219 °C.  $R_f=0.72$ . Anal calcd. for  $C_{28}H_{34}O_2$ : C, 83.54; H, 8.51. Found: 83.60; H, 8.42.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 0.96 (s, 3H, H-18), 1.31 (s, 9H, 4'-C(CH<sub>3</sub>)<sub>3</sub>), 6.69 (d,  $J=8.5$  Hz, 1H, H-2), 7.05 (m, 3H), 7.37 (m, 2H), 8.80 (s, 1H, 3-OH).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 20.9 (CH<sub>2</sub>), 24.9 (C-18), 28.3 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 31.7 (3C, 4'-C(CH<sub>3</sub>)<sub>3</sub>), 32.2 (CH<sub>2</sub>), 33.3 (CH<sub>2</sub>), 34.6 (4'-C(CH<sub>3</sub>)<sub>3</sub>), 40.9 (CH), 41.6 (CH), 49.0 (CH), 49.9 (C-13), 113.3 (C-2), 125.1 (2C, 2 $\times$ CH), 125.8 (C-1), 128.0 (C), 130.1 (2C, 2 $\times$ CH), 130.7 (C), 135.4 (C), 136.3 (C), 148.7 (C), 152.7 (C-3), 221.2 (C-17). MSI  $m/z$  (%): 403 (100, [M+H]<sup>+</sup>).

#### 2.1.2. Reaction conditions for Suzuki-Miyaura coupling in 3-OBn series

3-Benzyloxy-2-bromo-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**3b**, 220 mg, 0.50 mmol) or 3-Benzyloxy-2-bromo-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**6b**, 220 mg, 0.50 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (58 mg, 0.050 mmol, 0.1 equiv), K<sub>2</sub>CO<sub>3</sub> (276 mg, 2.0 mmol, 4 equiv.), (4-subst.)phenylboronic acid (2 equiv.) and toluene (4 ml) were added under nitrogen atmosphere, in 10 mL Pyrex pressure vessels (CEM, Part #: 908035) with silicone cap (CEM, Part #: 909210) and the mixture was heated in a CEM microwave reactor at 150 °C for 30 min under stirring. The solvent was evaporated in vacuo. The residue was purified by flash chromatography with 5% ethyl acetate/95% hexane as eluent.

##### 2.1.2.1. 3-Benzyloxy-2-phenyl-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**11b**)

Compound **11b** was isolated as white crystals (203 mg, 92%). Mp: 170–176 °C.  $R_f=0.70$ . Anal calcd. for  $C_{31}H_{32}O_2$ : C, 85.28; H, 7.39. Found: 85.36; H, 7.30.  $^1H$  NMR (C<sub>6</sub>D<sub>6</sub>)  $\delta$  ppm: 0.79 (s, 3H, H-18), 2.55–2.66 (overlapping multiplets, 2H, H-6), 4.79 (s, 2H, 3-OCH<sub>2</sub>), 6.63 (s, 1H, H-4), 7.01–7.32 (overlapping multiplets, 9H), 7.21 (d,  $J=7.4$  Hz, 2H),  $^{13}C$  NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 21.1 (CH<sub>2</sub>), 25.2 (C-18), 28.3 (CH<sub>2</sub>), 28.4 (CH<sub>2</sub>), 30.3 (CH<sub>2</sub>), 32.1 (CH<sub>2</sub>), 33.5 (CH<sub>2</sub>), 41.5

(CH), 41.6 (CH), 49.4 (CH), 50.1 (C-13), 70.7 (OCH<sub>2</sub>), 113.6 (C-4), 126.7 (CH), 126.9 (2×CH), 127.5 (CH), 127.9 (2×CH), 128.4 (2×CH), 128.7 (CH), 129.2 (2×CH), 129.6 (C), 132.6 (C), 137.2 (C), 137.5 (C), 138.8 (C), 153.7 (C), 221.4 (C-17). MSI *m/z* (%): 437 (100, [M+H]<sup>+</sup>).

#### 2.1.2.2. 3-Benzyloxy-2-(4-chlorophenyl)-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**12b**)

Compound **12b** was isolated as white crystals (221 mg, 94%). Mp.: 149–157°C. R<sub>f</sub>=0.67. Anal calcd. for C<sub>31</sub>H<sub>31</sub>ClO<sub>2</sub>: C, 79.05; H, 6.63. Found: 79.12; H, 6.54. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 1.06 (s, 3H, H-18), 2.86 (m, 2H, H-6), 5.02 (s, 2H, 3-OCH<sub>2</sub>), 6.74 (s, 1H, H-4), 7.21 (s, 1H, H-1), 7.28–7.34 (overlapping multiplets, 7H), 7.47 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 21.0 (CH<sub>2</sub>), 25.1 (C-18), 28.2 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 30.3 (CH<sub>2</sub>), 32.0 (CH<sub>2</sub>), 33.4 (CH<sub>2</sub>), 41.4 (CH), 41.5 (CH), 49.2 (CH), 50.1 (C-13), 70.5 (3-OCH<sub>2</sub>), 113.3 (C-4), 126.8 (2C, 2×CH), 127.6 (C-2), 127.7 (CH), 128.0 (2C, 2×CH), 128.4 (3C, 3×CH), 130.8 (2C, 2×CH), 132.5 (2C, 2×C), 137.1 (2C, C), 137.6 (C), 153.4 (C-3), 221.5 (C-17). MSI *m/z* (%): 472 (100, [M+H]<sup>+</sup>).

#### 2.1.2.3. 3-Benzyloxy-2-(4-*tert*-butylphenyl)-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**13b**)

Compound **13b** was isolated as white crystals (227 mg, 92%). Mp.: 163–166°C. R<sub>f</sub>=0. Anal calcd. for C<sub>35</sub>H<sub>40</sub>O<sub>2</sub>: C, 85.32; H, 8.18. Found: 85.40; H, 8.09. <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>)  $\delta$  ppm: 0.79 (s, 3H, H-18), 1.27 (s, 9H, 4'-C(CH<sub>3</sub>)<sub>3</sub>), 2.63 (m, 2H, H-6), 4.82 (s, 2H, 3-OCH<sub>2</sub>), 6.66 (s, 1H, H-4), 7.03 (t, *J* = 7.3 Hz, 1H, H-4''), 7.11 (t, *J* = 7.6 Hz, 2H, H-3'' and H-5''), 7.23 (d, *J* = 7.5 Hz, 2H, H-2'' and H-6''), 7.35 (s, 1H, H-1), 7.41 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 8.4 Hz, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 21.0 (CH<sub>2</sub>), 25.1 (C-18), 28.2 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 30.2 (CH<sub>2</sub>), 31.4 (3C, 4'-C(CH<sub>3</sub>)<sub>3</sub>), 32.1 (CH<sub>2</sub>), 33.4 (CH<sub>2</sub>), 34.5 (4'-C(CH<sub>3</sub>)<sub>3</sub>), 41.5 (2C, 2×CH), 49.2 (CH), 50.1 (C-13), 70.6 (3-OCH<sub>2</sub>), 113.5 (C-4), 124.8 (2C, 2×CH), 126.8 (2C, 2×CH), 127.4 (CH), 128.3 (2C, 2×CH), 128.6 (CH), 128.9 (C), 129.2 (2C, 2×CH), 132.4 (C), 135.6 (C), 136.8 (C), 137.5 (C), 149.4 (C), 153.7 (C-3), 221.6 (C-17). MSI *m/z* (%): 493 (100, [M+H]<sup>+</sup>).

#### 2.1.2.4. 3-Benzyloxy-4-phenyl-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**14b**)

Compound **14b** was isolated as white crystals (196 mg, 90%). Mp.: 58–60 °C. R<sub>f</sub>=0.72. Anal calcd. for C<sub>31</sub>H<sub>32</sub>O<sub>2</sub>: C, 85.28; H, 7.39. Found: 85.35; H, 7.31. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 1.05 (s, 3H, H-18), 4.95 (d, *J* = 3.9 Hz, 2H, 3-OCH<sub>2</sub>), 6.85 (d, *J* = 8.7 Hz, 1H, H-2), 7.08 (s, 1H, H-1), 7.11 (m, 2H), 7.19–7.25 (overlapping multiplets, 6H), 7.33 (m, 1H), 7.41 (m, 1H). <sup>13</sup>C NMR

(CDCl<sub>3</sub>)  $\delta$  ppm: 21.1 (CH<sub>2</sub>), 25.1 (C-18), 28.4 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 32.1 (CH<sub>2</sub>), 33.4 (CH<sub>2</sub>), 41.0 (CH), 41.8 (CH), 49.5 (CH), 50.1 (C-13), 70.5 (3-OCH<sub>2</sub>), 111.1 (C-2), 125.7 (CH), 126.5 (2C, CH), 126.6 (CH), 127.3 (CH), 128.0 (2C, 2 $\times$ CH), 128.2 (2C, 2 $\times$ CH), 129.9 (CH), 130.1 (CH), 131.2 (C), 133.1 (C), 136.6 (C), 137.6 (C), 137.8 (C), 153.9 (C-3), 221.4 (C-17). MSI  $m/z$  (%): 437 (100, [M+H]<sup>+</sup>).

#### 2.1.2.5. 3-Benzyloxy-4-(4-chlorophenyl)-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**15b**)

Compound **15b** was isolated as white crystals (224 mg, 95%). Mp.: 67–69°C. R<sub>f</sub>=0.74. Anal calcd. for C<sub>31</sub>H<sub>31</sub>ClO<sub>2</sub>: C, 79.05; H, 6.63. Found: 79.11; H, 6.56. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 1.04 (s, 3H, H-18), 4.96 (d,  $J$ = 4.5 Hz, 2H, 3-OCH<sub>2</sub>), 6.83 (d,  $J$ = 8.7 Hz, 1H, H-2), 7.12 (m, 3H), 7.11–7.25 (overlapping multiplets, 4H), 7.28 (m, 1H), 7.37 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 21.0 (CH<sub>2</sub>), 25.0 (C-18), 28.3 (CH<sub>2</sub>), 28.4 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 32.1 (CH<sub>2</sub>), 33.4 (CH<sub>2</sub>), 40.9 (CH), 41.7 (CH), 49.3 (CH), 50.1 (C-13), 70.2 (3-OCH<sub>2</sub>), 110.8 (C-2), 126.1 (CH), 126.5 (2C, 2 $\times$ CH), 127.4 (CH), 128.2 (2C, 2 $\times$ CH), 128.3 (2C, 2 $\times$ CH), 129.6 (C), 131.3 (CH), 131.5 (CH), 132.5 (C), 133.0 (C), 136.2 (C), 136.5 (C), 137.4 (C), 153.7 (C-3), 221.6 (C-17). MSI  $m/z$  (%): 494 (100, [M+Na]<sup>+</sup>).

#### 2.1.2.6. 3-Benzyloxy-4-(4-*tert*-butylphenyl)-13 $\alpha$ -estra-1,3,5(10)-trien-17-one (**16b**)

Compound **16b** was isolated as white crystals (222 mg, 90%). Mp.: 66–70°C. R<sub>f</sub>=0.76. Anal calcd. for C<sub>35</sub>H<sub>40</sub>O<sub>2</sub>: C, 85.32; H, 8.18. Found: 85.41; H, 8.10. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 1.05 (s, 3H, H-18), 1.38 (s, 9H, 4'-C(CH<sub>3</sub>)<sub>3</sub>), 4.93 (d,  $J$ = 6.5 Hz, 2H, 3-OCH<sub>2</sub>), 6.85 (d,  $J$ = 8.6 Hz, 1H, H-2), 7.06 (m, 2H), 7.14 (m, 1H), 7.21 (m, 5H), 7.43 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 21.0 (CH<sub>2</sub>), 25.0 (C-18), 28.4 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 31.4 (3C, 4'-C(CH<sub>3</sub>)<sub>3</sub>), 32.1 (CH<sub>2</sub>), 33.4 (CH<sub>2</sub>), 34.5 (4'-C(CH<sub>3</sub>)<sub>3</sub>), 40.9 (CH), 41.8 (CH), 49.4 (CH), 50.1 (C-13), 70.5 (3-OCH<sub>2</sub>), 111.3 (C-2), 124.8 (2C, 2 $\times$ CH), 125.5 (CH), 126.5 (2C, 2 $\times$ CH), 127.2 (CH), 128.1 (3C, 3 $\times$ CH), 129.4 (CH), 129.6 (CH), 131.3 (C), 134.6 (CH), 136.8 (C), 137.6 (C), 149.3 (C), 154.1 (C-3), 221.7 (C-17). MSI  $m/z$  (%): 493 (100, [M+H]<sup>+</sup>).

## 2.2. Determination of antiproliferative activities

The antiproliferative properties of the newly synthesized compounds (**3**, **6** and **11–16**) were determined on a panel of human adherent cancer cell lines of gynecological origin. MCF-7 and MDA-MB-231 were isolated from breast cancers differing in biochemical background, while A2780 cells were isolated from ovarian cancer. Hela and SiHa are cervical cancer cell lines



positive for HPV-18 and HPV-16, respectively. The cancer selectivity of compounds was tested on the non-cancerous mouse embryo fibroblast cell line NIH/3T3. All cell lines were purchased from European Collection of Cell Cultures (ECCAC, Salisbury, UK) exception for Siha (American Tissue Culture Collection, Manassas, VA, USA). Cells were cultivated in minimal essential medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic–antimycotic mixture. All media and supplements were obtained from Lonza Group Ltd., Basel, Switzerland. Near-confluent cancer cells were seeded onto a 96-well microplate (5000 cells/well) and, after overnight standing, 200  $\mu$ L new medium, containing the tested compounds at 10 and 30  $\mu$ M, was added. After incubation for 72 h at 37 °C in humidified air containing 5% CO<sub>2</sub>, the living cells were assayed by the addition of 20  $\mu$ L of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution. MTT was converted by intact mitochondrial reductase and precipitated as purple crystals during a 4-h contact period. The medium was next removed and the precipitated formazan crystals were dissolved in 100  $\mu$ L of DMSO during a 60-min period of shaking at 37 °C.

Finally, the reduced MTT was assayed at 545 nm, using a microplate reader utilizing wells with untreated cells serving as control [31]. In the case of the most active compounds (i.e. higher than 50% growth inhibition at 30  $\mu$ M), the assays were repeated with a set of dilutions, sigmoidal dose–response curves were fitted to the determined data and the IC<sub>50</sub> values (the concentration at which the extent of cell proliferation was half that of the untreated control) were calculated by means of GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). All in vitro experiments were carried out on two microplates with at least five parallel wells. Stock solutions of the tested substances (10 mM) were prepared in DMSO. The highest DMSO content of the medium (0.3%) did not have any substantial effect on cell proliferation. Cisplatin (Ebewe Pharma GmbH, Unterach, Austria) was used as positive control.

### 3. Results and discussion

The aromatic ring of 13 $\alpha$ -estrone might readily be halogenated using different types of halogenating agents (Scheme 1, [8]). *N*-Halosuccinimides are suitable for the non-selective transformations of the phenolic moiety. Due to the activated behavior of ring A, and the *ortho*-directing ability of the 3-OH group, a mixture of *mono* (2- or 4-substituted) and *bis* (2,4-disubstituted) derivatives is formed. We demonstrated recently that the chemo- and regioselectivity of halogenations depend on the reagent and solvent applied [8]. Additionally, the nature and the size of the functional group at C-3 also influence the outcome of the reactions. Bis-2,4-derivates (beside the monosubstituted compounds) were formed exclusively when the starting compound possessed a phenolic OH group. In the case of methyl or benzyl ethers, only monosubstitutions occurred. We showed recently that 13 $\alpha$ -estrone derivatives halogenated at ring A represent a very promising compound class with diverse biological activities. Enzymes 17 $\beta$ -hydroxysteroid dehydrogenase 1 (17 $\beta$ -HSD1 and steroid sulfatase (STS) involved in estradiol biosynthesis could effectively be inhibited by certain halo derivatives. The 3-hydroxy-2,4-*bis*-iodo derivative proved to be a dual inhibitor. The 2-bromo-3-hydroxy derivative should also be highlighted as it also displayed dual inhibitory properties against 17 $\beta$ -HSD1 enzyme and OATP2B1 transporter [8,13]. These favorable biological properties of compounds halogenated at ring A inspired us to perform further synthetic transformations using the compounds as aryl halide substrates. First, C(sp<sup>2</sup>)-C(sp) couplings were carried out in order to introduce large, but apolar groups onto the C-2 and C-4 positions. Sonogashira couplings with phenylacetylenes in the 3-OH series resulted in new compounds with substantial 17 $\beta$ -HSD1 inhibitory potentials [9]. Products in the C-2 series, in turn, exhibited exclusive inhibitory potentials. These results suggest that the nature of the substituents at C-2, C-3 and/or C-4 exerts a great impact on the biological activity of 13 $\alpha$ -estrone derivatives.

Scheme 1.

13 $\alpha$ -Estrone was diversified at several sites, including different modifications at C-3 and/or in ring D with the aim of getting new compounds with potential antiproliferative behavior. It was shown that certain derivatives possess substantial cell growth-inhibitory potential against a number of human cancer cell lines of reproductive origin. We proved in many cases, that the presence of the apolar, bulky benzyl ether function at position C-3 is markedly beneficial concerning the antiproliferative potential [14,15,17].

It follows that 3-OH compounds might have increased enzyme inhibitory properties, whereas 3-OBn derivatives might be promising cytostatic candidates. Nevertheless, it is worth evaluating new compounds in both series in order to get an insight into the correlation between activity and structure.

Based on these findings, we selected 13 $\alpha$ -estrone and its 3-*O*-benzyl counterpart as starting materials. The latter was synthesized in one step from estrone 3-benzyl ether in reaction with *ortho*-phenylenediamine in acetic acid. The Pd/C-catalyzed hydrogenolysis of the 3-benzyl-13 $\alpha$ -estrone product resulted in 13 $\alpha$ -estrone in high yield. The halogenation of 3-OH and 3-OBn substrates was achieved using *N*-halosuccinimides as described earlier [8]. Three different electrophile triggers (NCS, NBS or NIS) were used for the formation of aryl halides in order to investigate the influence of the nature of the halogen substituent on the C–C coupling reaction. The monosubstituted regioisomers (**2a,b–7a,b**) served as starting compounds in Suzuki–Miyaura couplings (Scheme 2).

## Scheme 2

Phenylboronic acid and its two 4-substituted derivatives were chosen as coupling partners. 4-Chlorophenylboronic acid seemed to be interesting due to the dual (negative inductive but positive mesomeric) electronic properties of chlorine. The large alkyl substituent in 4-*tert*-butylphenylboronic acid might greatly influence the biological behavior as earlier established [15]. The reaction conditions applied here have been chosen according to our coupling methods described recently. Pd(PPh<sub>3</sub>)<sub>4</sub> was used as catalyst, K<sub>2</sub>CO<sub>3</sub> as base, and transformations have been carried out under microwave irradiation. First we performed C–C couplings of substrate **2a,b–7a,b** with phenylboronic acid as a model reagent (Table 1). 2-Substituted regioisomers in the 3-OH series (**3a**, **4a**) could efficiently be transformed (Table 1, Entries 2 and 3), except for the 2-chloro derivative (**2a**, Table 1, Entry 1). The latter could only be coupled using KO<sup>*t*</sup>-Bu as base at elevated temperature (150 °C) and longer microwave irradiation (60 min) (Table 1, Entry 13). These conditions proved to be applicable for the transformations of 4-chloro-3-hydroxy (**5a**) and 3-benzyloxy-2- or 4-chloro derivatives (**2b** and **5b**), too (Table 1, Entries 14–16). Concerning the reactions of bromo or iodo compounds (**3**, **4**, **6**, and **7**), these substrates behaved in a similar way in these reactions (irrespective of the regioisomerism) (Table 1, Entries 2, 3, 5, 6, 8, 9, 11, 12). However, pronounced dehalogenation were observed in the case of iodo starting compounds (Table 1, Entries 3, 6, 9, 12). The nature of the substituent at C-3 of 13 $\alpha$ -estrane core influenced the outcome of couplings as established earlier in Hirao reactions

[10]. Benzyl ethers needed harsher conditions (higher reaction temperature) than their hydroxy counterparts, due to stronger steric hindrance (Table 1, Entries 8, 9, 11, 12).

Table 1

After establishing the appropriate reaction conditions (Table 1, Entries 2 and 8), 2- or 4-bromo-3-OH and -3-OBn derivatives (**3a,b** or **6a,b**) have been chosen for further transformations (Scheme 3). Suzuki–Miyaura couplings with subst.-phenylboronic acids afforded the desired products (**11–16**) in excellent yields. 12 new arylated compounds have been synthesized. The structure of the newly synthesized 13 $\alpha$ -estrone derivatives (**11–16**) have been deduced from  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra.

Scheme 3

The antiproliferative properties of the phenylated compounds (**11a,b–16a,b**) and their direct precursors (**3a,b** and **6a,b**) were determined *in vitro* on a panel of human adherent breast (MCF-7 and MDA-MB-231), cervical (HeLa and SiHa), and ovarian (A2780) cancer cell lines by means of MTT assay (Table 2). The two breast cancer cell lines involved in this study have different receptorial status [32–34]. In contrast with the estrogen receptor positive MCF-7 cell line, MDA-MB-231 is a triple-negative cell line lacking all current clinically utilized pharmacological targets (receptors for estrogens, progesterone, and growth factor HER2/neu) for hormonal treatment. The triple-negative subtype of breast cancer, therefore, represents the portion of poorest prognosis among breast cancer cases [35]. The majority of cervical cancers is associated with human papilloma virus (HPV) infection [36,37]. Despite vaccination and screening, cervical cancer still causes high mortality rate in less developed countries. The utilized cell lines Hela and Siha represent HPV-18 and -16 positive cases, respectively. These two types of HPV are responsible for nearly 70% of cervical cancerous cases. Ovarian cancer is another common type of gynecological malignancies [38]. Its poor prognosis and high mortality rates arise from its high recurrence and acquired resistance to drug treatment. In order to investigate the antiproliferative properties of the synthesized compounds, the ovarian cancer cell line A2780 was also included. Evaluation of the promising antiproliferative compounds on

the mentioned cell lines might initiate the development of new anticancer agents acting with better tolerance and higher effectiveness.

Our data reveal conclusive structure–activity correlations concerning the bromo derivatives (**3a,b** and **6a,b**, Table 2). The 3-OH compounds (**3a** and **6a**) did not influence the cell growth of the tested tumor cells markedly. However, the 4-regioisomer **6b** of benzyl ethers exerted outstanding antiproliferative effect against all investigated cell lines. MCF-7 cells were the most sensitive to **6b** with IC<sub>50</sub> value in a submicromolar range. Compound **6b** exerted somewhat lower inhibition against the cervical cell lines Hela and SiHa and triple negative breast cancer MDA-MB-231 with IC<sub>50</sub> values in the low micromolar range. It is worth mentioning that **6b** made a marked distinction between the two breast cancer cell lines, but not between the two cervical ones. The other 3-OBn regioisomer (**3b**) exhibited weak antiproliferative effect on the tested cell lines. As concerns compounds **3b** and **6b**, it should be highlighted, that substantial dependence of the regioselectivity on the antiproliferative potential was observed (in favor of the 4-regioisomer). The most relevant compounds were additionally tested against a mouse fibroblast cell line (NIH-3T3) in order to obtain preliminary data concerning the cancer selectivity of the elicited action. Compound **6b** exerted only a limited (less than 50% inhibition even at 30 µM) action on this cell line, though the cells are highly sensitive to reference agent cisplatin.

A comparison of the data for phenylated 3-OH (**11a** and **14a**) and brominated 3-OH derivatives (**3a** and **6a**) reveals that the presence of the phenyl ring instead of a bromine improved the antiproliferative activities only for the 2-regioisomer. The calculated IC<sub>50</sub> values for compound **11a** were over 10 micromoles against MCF-7 and Hela, and the effects were weaker on the other cell lines including mouse fibroblasts. **11a** proved to be more effective than **14a** its 4-counterpart. A moderate activity enhancement was observed in the case of **11b**, which might be attributed to the presence of the 3-OBn function. Strikingly, the phenylated 4-regioisomer (**14b**) was less effective than its 2-counterpart (**11b**), and far less potent than its bromo derivative **6b**.

Setting against the results for phenyl and (subst.)phenyl derivatives demonstrates that the nature and the size of the ring A substituents greatly influence cytostatic properties.

In general, compounds **13a** and **16a** bearing the 4"-*t*-Bu function seemed to be more potent than **11a** and **14a**. The 3-OBn-4"-*t*-Bu derivatives (**13b** and **16b**) displayed very weak growth inhibition, irrespective of the substitution pattern of ring A.

Substantial enhancements in cytostatic potential were detected for the 2-(4"-chlorophenyl) 3-OH compound (**12a**), except for A2780 cell line. **12a** exerted pronounced effect on Hela cells

with a 3.33  $\mu\text{M}$   $\text{IC}_{50}$  value, which is comparable with that of the most potent compound **6b**. The next lowest  $\text{IC}_{50}$  value was obtained on MCF-7 for compound **12a**, but it is far behind the corresponding data of **6b**. It is important to note that essential differences arose here in the inhibitory values for the breast and cervical cell line pairs. This was not the case against Hela and SiHa concerning the results of **6b**. The activity dependence of regioisomerism here was also significant, but the tendency was reversed to that in the case of **3b** and **6b**. Here the 2-isomer (**12a**) was far more potent than its 4-counterpart (**15a**). The latter does not seem to be true for the (4"-chlorophenyl)-3-OBn compounds (**12b** and **15b**), since **15b** exerted more pronounced cell growth inhibitory effect against all cell lines. This is in a good agreement with the data obtained for **3b** and **6b**, but the potency of **15b** is appreciably lower.

Our 2-phenylethynyl derivative (**I**) synthesized recently was additionally included in the investigations. We were interested in comparison of the antiproliferative properties of 2-phenyl (**11a**) and 2-phenylethynyl (**I**) derivatives. The data in Table 2 reflect that the moderate activity of **11a** decreased substantially, when a short linear linker ( $\text{C}\equiv\text{C}$ ) is inserted between the two phenyl groups. Accordingly, the nature and geometry of the connection might substantially influence the growth-inhibitory properties of the cross-coupled compounds.

Table 2

#### 4. Conclusion

In conclusion, 2- or 4-[(subst.) phenyl]-13 $\alpha$ -estrone derivatives have been synthesized via efficient, microwave-induced, Pd-catalyzed Suzuki–Miyaura coupling. Reactions of 2- or 4-halogenated 13 $\alpha$ -estrone derivatives with (4-subst.)phenylboronic acids using Pd(PPh<sub>3</sub>)<sub>4</sub> as catalyst afforded the desired phenylated products in high yields. Certain effective submicromolar or low micromolar cell growth inhibitors (**6b**, **12a**, and **13a**) have been identified against human cancer cell lines of gynecological origin. The estrogen receptor positive MCF-7 and the HPV-18 positive Hela cancer cell lines were the most sensitive to the tested compounds. None of the most promising agents exerted substantial growth inhibitory action against fibroblasts indicating the cancer selectivity of the action. The most potent compound (**6b**) exerted outstanding submicromolar antitumoral activity against MCF-7 cell line and lower calculated IC<sub>50</sub> values against all cell lines in comparison to those of reference agent cisplatin. The most effective phenylated compound (**12a**) exerted selective low micromolar activities against two cell lines (MCF-7 and Hela). Compounds **6b** and **12a** behaved similarly as concerns breast cell lines MCF-7 and MDA-MB-231. However, **12a** differentiated between the two cervical cell lines Hela and SiHa. Accordingly, ring A substitution of 13 $\alpha$ -estrone derivatives leads to effective antitumoral compounds. The substitution pattern of the aromatic ring influences cell growth inhibition markedly. The promising inhibitory data motivate further structural modifications in ring A of 13 $\alpha$ -estrone, and additional investigations on the mechanism of antiproliferative action.

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## Legends for Tables, Figures and Schemes

Table 1: Effect of reaction conditions on C–C coupling of halogenated 13 $\alpha$ -estrone (**2a,b**–**7a,b**) with phenylboronic acid in toluene catalyzed by Pd(PPh<sub>3</sub>)<sub>4</sub><sup>1</sup>

Table 2: Antiproliferative properties of the synthesized compounds

Figure 1: The structure of 13 $\alpha$ -estrone (**1a**) and its 3-benzyl ether (**1b**)

Figure 2: Potent, biologically active 13 $\alpha$ -estrone derivatives (**I**, **3a** and **II**)

Scheme 1: Synthesis of ring A halogenated 13 $\alpha$ -estrone derivatives

Scheme 2: Suzuki-Miyaura couplings with phenylboronic acid as model reagent

Scheme 3: Suzuki–Miyaura couplings of 2- or 4-bromo-13 $\alpha$ -estrone. Reactions were performed on a 0.50 mmol scale with 2 equiv of subst.-phenylboronic acid, 0.1 equiv. Pd(PPh<sub>3</sub>)<sub>4</sub>, 4 equiv. K<sub>2</sub>CO<sub>3</sub>, at 100 °C, 30 min under microwave irradiation.

## Footnotes

Table 1:

<sup>1</sup> Aryl halide (0.50 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.1 equiv), K<sub>2</sub>CO<sub>3</sub> (4 equiv.), (4-subst.)phenylboronic acid (2 equiv.), toluene, N<sub>2</sub> atmosphere.

<sup>2</sup> Flash chromatography yield obtained under microwave irradiation.

Table 2:

<sup>1</sup> Mean value from two independent measurements with five parallel wells; standard deviation <20%. n.t.: not tested.

<sup>2</sup> Inhibition values <20% are not presented

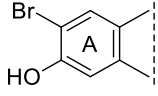
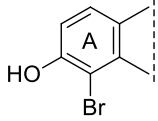
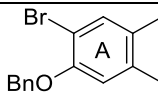
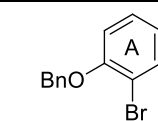
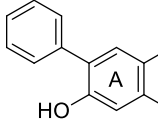
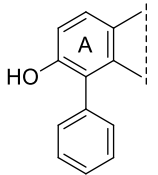
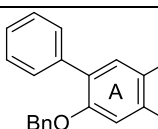
Table 1.

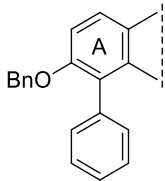
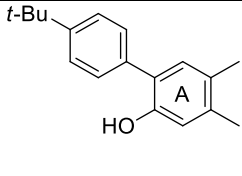
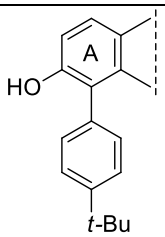
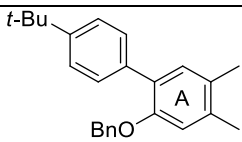
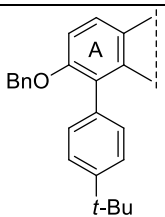
Entry	Substrate	Base	Temp (°C)	Reaction time (min)	Products <sup>2</sup> (%)
1	<b>2a</b>	K <sub>2</sub> CO <sub>3</sub>	100	30	0
2	<b>3a</b>	K <sub>2</sub> CO <sub>3</sub>	100	30	<b>11a + 1a</b> (95 + 2)
3	<b>4a</b>	K <sub>2</sub> CO <sub>3</sub>	100	30	<b>11a + 1a</b> (83 + 12)
4	<b>5a</b>	K <sub>2</sub> CO <sub>3</sub>	100	30	0
5	<b>6a</b>	K <sub>2</sub> CO <sub>3</sub>	100	30	<b>14a + 1a</b> (94 + 3)
6	<b>7a</b>	K <sub>2</sub> CO <sub>3</sub>	100	30	<b>14a + 1a</b> (80 + 15)
7	<b>2b</b>	K <sub>2</sub> CO <sub>3</sub>	150	30	0
8	<b>3b</b>	K <sub>2</sub> CO <sub>3</sub>	150	30	<b>11b + 1b</b> (92 + 4)
9	<b>4b</b>	K <sub>2</sub> CO <sub>3</sub>	150	30	<b>11b + 1b</b> (82 + 7)
10	<b>5b</b>	K <sub>2</sub> CO <sub>3</sub>	150	30	0
11	<b>6b</b>	K <sub>2</sub> CO <sub>3</sub>	150	30	<b>14b + 1b</b> (90 + 4)
12	<b>7b</b>	K <sub>2</sub> CO <sub>3</sub>	150	30	<b>14b + 1b</b> (81 + 10)
13	<b>2a</b>	KO <i>t</i> -Bu	150	60	<b>11a</b> (43)
14	<b>5a</b>	KO <i>t</i> -Bu	150	60	<b>14a</b> (51)
15	<b>2b</b>	KO <i>t</i> -Bu	150	60	<b>11b</b> (41)
16	<b>5b</b>	KO <i>t</i> -Bu	150	60	<b>14b</b>

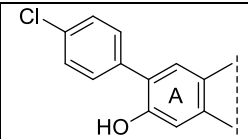
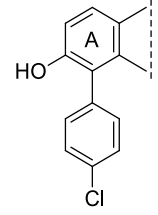
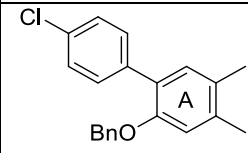
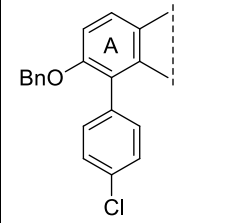
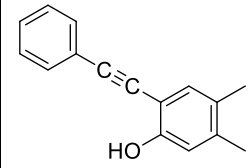


					(40)
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Table 2.

Comp.	Comp. number	Conc. ( $\mu$ M)	Inhibition (%) $\pm$ SEM [Calculated IC <sub>50</sub> ] <sup>1</sup>					
			MCF-7	MDA-MB-231	HeLa	SiHa	A2780	NIH-3T3
	<b>3a</b>	10	-	-	-	-	-	n.t.
		30	24.26 $\pm$ 0.76	22.85 $\pm$ 1.97	32.26 $\pm$ 0.52	20.72 $\pm$ 3.56	-	
	<b>6a</b>	10	-	-	-	-	-	n.t.
		30	51.92 $\pm$ 1.61	42.09 $\pm$ 1.31	54.81 $\pm$ 1.14	-	44.21 $\pm$ 1.09	
	<b>3b</b>	10	-	-	-	-	-	n.t.
		30	-	-	48.57 $\pm$ 1.40	-	-	
	<b>6b</b>	10	76.67 $\pm$ 0.30	54.41 $\pm$ 0.88	60.23 $\pm$ 0.42	59.04 $\pm$ 1.51	47.78 $\pm$ 2.10	30.59 $\pm$ 0.47
		30	79.45 $\pm$ 0.85 (0.52)	62.36 $\pm$ 0.67 (3.91)	68.16 $\pm$ 0.62 (2.85)	63.04 $\pm$ 0.41 (2.74)	54.75 $\pm$ 1.06	29.55 $\pm$ 0.36
	<b>11a</b>	10	44.72 $\pm$ 0.73	31.50 $\pm$ 1.77	36.82 $\pm$ 0.98	33.49 $\pm$ 0.90	35.73 $\pm$ 1.21	35.48 $\pm$ 1.33
		30	71.54 $\pm$ 0.40 (13.12)	48.97 $\pm$ 0.42	63.67 $\pm$ 0.30 (17.06)	54.36 $\pm$ 0.37	45.89 $\pm$ 0.96	40.04 $\pm$ 1.48
	<b>14a</b>	10	-	-	-	-	-	n.t.
		30	-	-	27.29 $\pm$ 1.02	-	-	
	<b>11b</b>	10	32.11 $\pm$ 1.90	-	51.26 $\pm$ 0.20	25.45 $\pm$ 2.09	25.44 $\pm$ 1.06	-
		30	74.50 $\pm$ 1.16 (16.87)	76.16 $\pm$ 1.05 (18.92)	75.53 $\pm$ 0.18 (8.98)	70.06 $\pm$ 1.48 (18.56)	73.42 $\pm$ 0.41 (18.62)	-

	<b>14b</b>	10	-	-	-	-	-	n.t.
		30	$27.35 \pm 1.36$	-	-	-	$27.14 \pm 0.94$	
	<b>13a</b>	10	$47.97 \pm 0.98$	$42.24 \pm 1.02$	$66.29 \pm 0.87$	$44.03 \pm 1.15$	$52.46 \pm 0.20$	$45.30 \pm 2.71$
		30	$66.68 \pm 0.71$ (12.82)	$53.23 \pm 0.35$	$77.67 \pm 0.42$ (6.69)	$51.95 \pm 0.78$	$68.13 \pm 0.55$ (9.41)	$51.68 \pm 1.96$
	<b>16a</b>	10	-	-	$38.70 \pm 1.67$	-	$35.83 \pm 1.92$	-
		30	$96.93 \pm 0.33$ (15.73)	$91.02 \pm 0.59$ (17.19)	$97.96 \pm 0.15$ (11.19)	$92.43 \pm 0.88$ (16.90)	$97.93 \pm 0.17$ (10.38)	-
	<b>13b</b>	10	-	-	$24.51 \pm 1.62$	-	-	n.t.
		30	$28.21 \pm 0.85$	$34.85 \pm 0.52$	$52.36 \pm 1.25$	$20.00 \pm 0.79$	$64.77 \pm 1.82$	
	<b>16b</b>	10	-	-	-	-	-	n.t.
		30	$32.81 \pm 1.33$	-	-	-	$24.65 \pm 2.81$	
	<b>12a</b>	10	$53.41 \pm 0.23$	$25.95 \pm 0.43$	$73.65 \pm 0.22$	$50.85 \pm 0.30$	$24.97 \pm 0.52$	$26.47 \pm 1.33$

		30	$59.51 \pm 0.22$ (5.33)	$61.92 \pm 0.67$ (18.32)	$75.46 \pm 0.81$ (3.33)	$53.45 \pm 0.95$	$33.61 \pm 0.74$	$28.17 \pm 0.25$
	<b>15a</b>	10	-	-	-	-	$20.20 \pm 1.13$	-
		30	$88.41 \pm 1.35$ (19.76)	$52.83 \pm 1.31$	$80.24 \pm 0.21$ (17.91)	$72.66 \pm 1.09$ (21.19)	$82.92 \pm 0.45$ (14.97)	$20.9 \pm 0.42$
	<b>12b</b>	10	-	-	-	-	-	n.t.
		30	$36.29 \pm 1.21$	$22.91 \pm 0.55$	$56.17 \pm 1.12$	$47.26 \pm 0.35$	$34.63 \pm 0.48$	
	<b>15b</b>	10	$48.56 \pm 0.32$	-	$47.73 \pm 1.65$	-	-	-
		30	$77.13 \pm 0.40$ (9.95)	$33.67 \pm 1.39$	$64.12 \pm 2.00$	$62.32 \pm 0.94$	$55.04 \pm 0.61$	-
	<b>I</b>	10	$23.51 \pm 1.95$	-	-	-	-	n.t.
		30	$69.00 \pm 0.32$ (21.51)	$29.66 \pm 0.59$	$59.89 \pm 1.16$	$46.18 \pm 0.14$	$78.34 \pm 0.12$ (20.82)	
Cisplatin		10	$53.03 \pm 2.29$	-	$42.61 \pm 2.33$	$88.64 \pm 0.50$	$83.57 \pm 1.21$	$91.80 \pm 0.39$
		30	$86.90 \pm 1.22$ (5.78)	$71.47 \pm 1.20$ (19.13)	$99.93 \pm 0.26$ (12.43)	$90.18 \pm 1.78$ (7.84)	$95.02 \pm 0.28$ (1.30)	$93.68 \pm 0.20$ (2.70)

## Figures

Figure 1

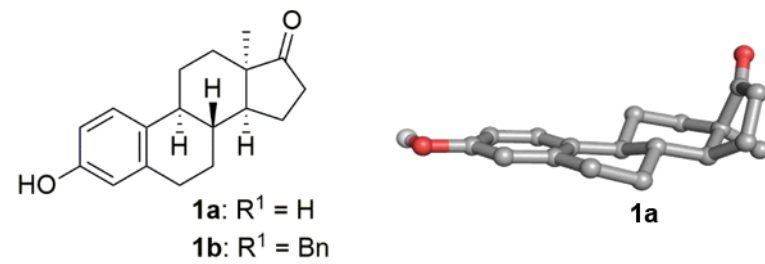
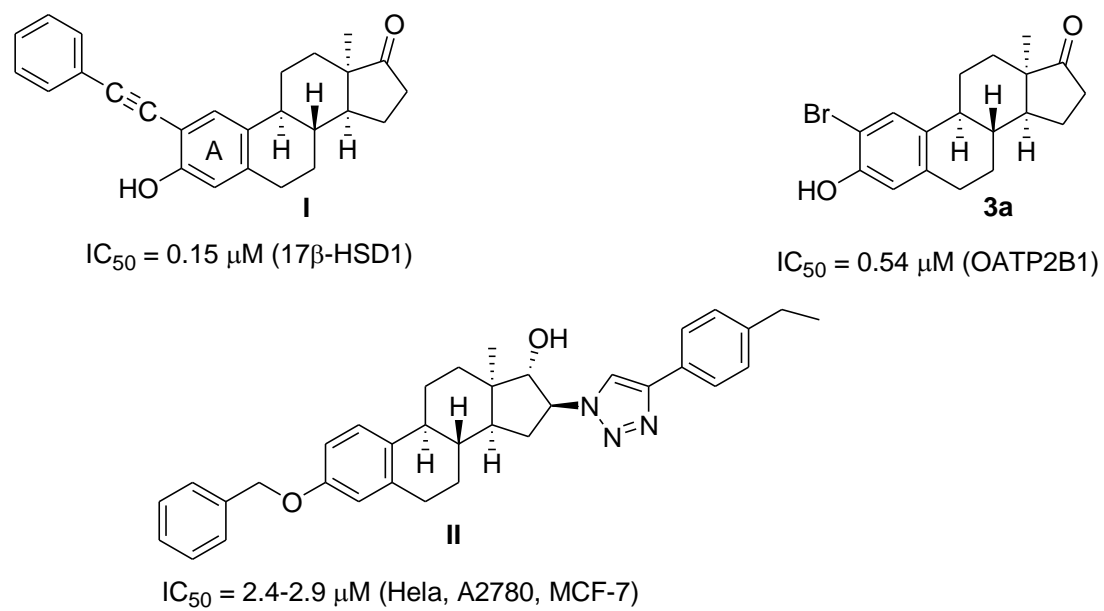
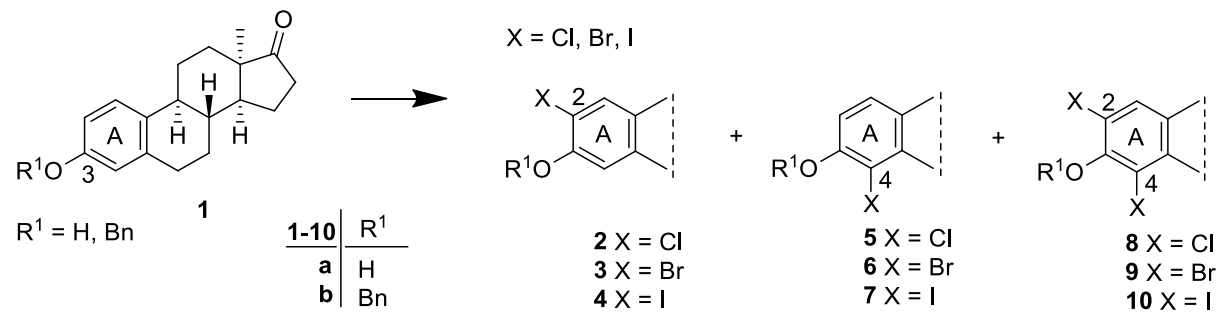


Figure 2

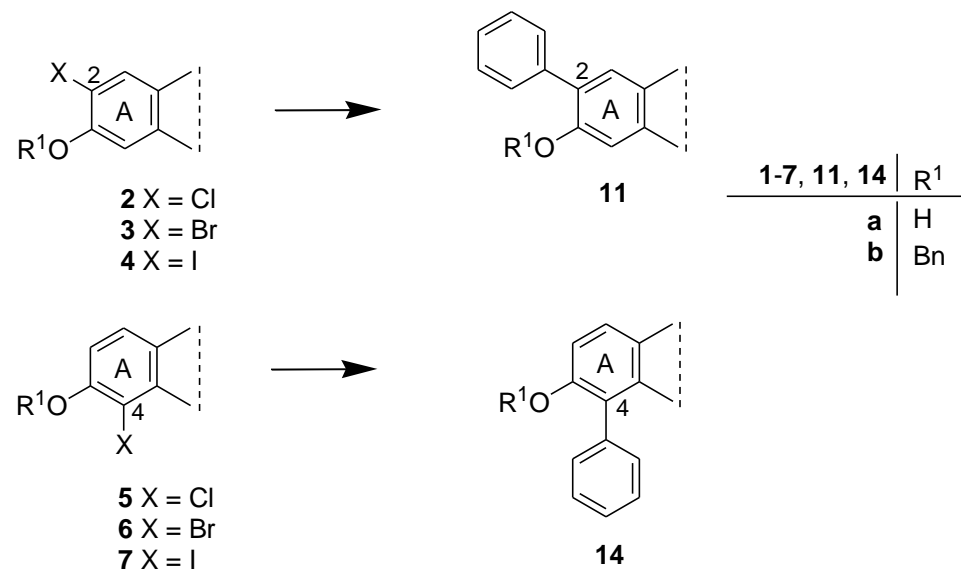


# Schemes

## Scheme 1



## Scheme 2



## Scheme 3

