Regioselective monoalkylation of 17β-estradiol for the synthesis of cytotoxic estrogens

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Abstract:

The regioselective synthesis of estrogens and their derivatives continues to be of interest. Most reported syntheses require multistep protocols associated with poor overall yield and lack of regioselectivity. New preparative protocols are still desired. Herein, 11 2-alkylated 17β-estradiol analogs were synthesized in a highly regioselective manner. The products were obtained using a convenient, one pot and high-yielding protocol. The anti-proliferative activity of the compounds was tested in human T-cell leukemia (CEM), human cervix carcinoma (HeLa) and human dermal microvascular endothelial (HMEC-1) cells.

Keywords: estrogens; estradiol; Friedel-Crafts reaction; regioselective alkylations: cytotoxicity; anti-cancer.

1. Introduction

The estrogen 2-methoxyestradiol (2-ME, **1**) was long believed to be an inactive endogenous metabolite of 17β-estradiol (**2**). However, in 1994, D´Amato, Folkman and co-workers showed that 2-ME (**1**) inhibits tubulin polymerization by interfering with the colchicine binding-site [1, 2]. Later studies revealed that the steroid **1** also blocks endothelial cell migration and proliferation *in vitro* [3]. Moreover, when administered to tumor-bearing mice, 2-ME (**1**) inhibited the vascularization and growth of solid tumors [2, 4]. In addition, it was reported that 2-ME (**1**) downregulates hypoxia-inducible factor-1α (HIF-1α) resulting in potent cytotoxic effects in prostate and breast cancer cells [5]. These observations were confirmed by *in vivo* studies that also showed that intrinsic and extrinsic apoptotic pathways were mediated by 2-ME (**1**) and its metabolites [6, 7]. 2-Methoxyestradiol (**1**) has entered several clinical trials that revealed no severe toxic effects, even when doses as high as 3 grams per day were administrated [8-11].

The aforementioned pharmacological activities have inspired several studies where 2-ME (**1**) has been used as a lead compound for the development of new anti-cancer agents [12]. Some examples of such analogs are depicted in Figure 1.

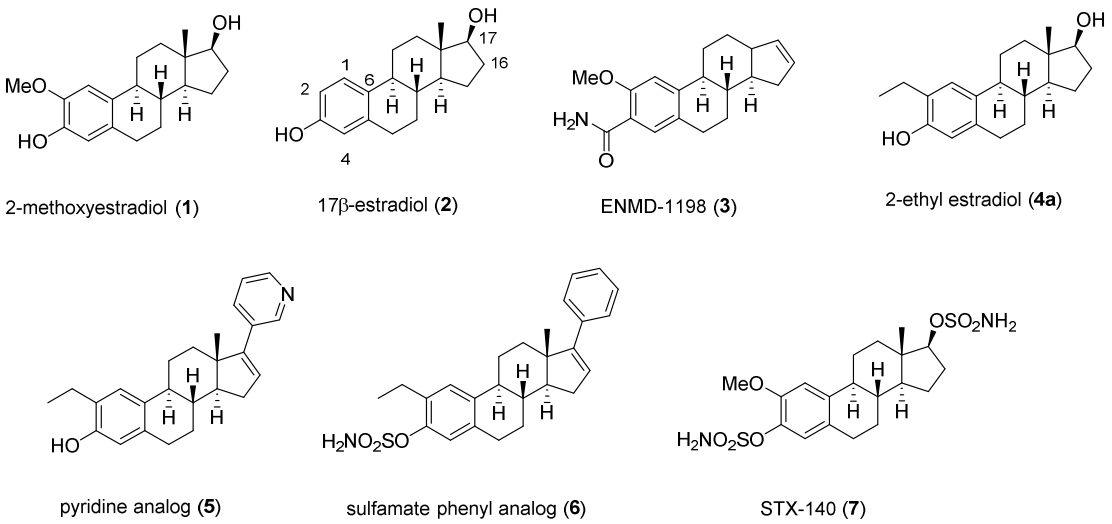
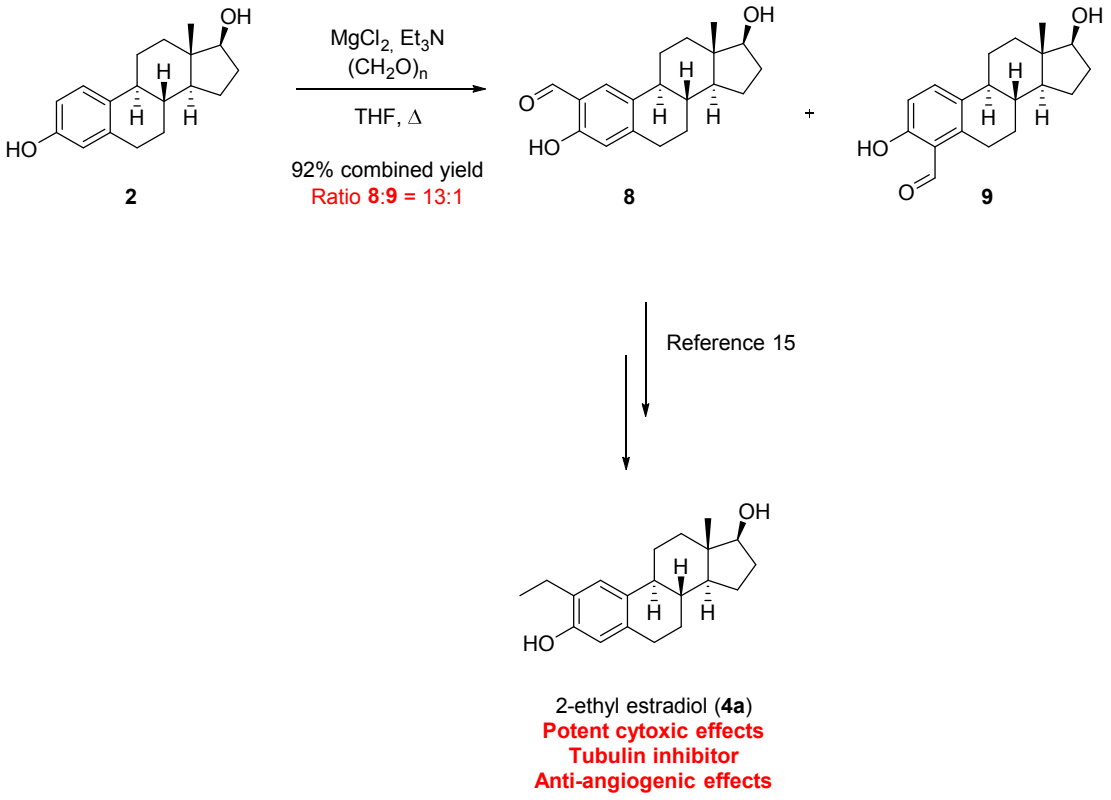


Fig. 1. Analogs of 2-ME (**1**) with anti-cancer activities.

We have previously used the steroid **1** as a lead compound for the synthesis of potential new anti-cancer agents [13-15]. The alkyl-substituent at C-2 seems interesting to alter in further efforts. The classic Friedel-Crafts reaction is the method

of choice for the introduction of secondary or tertiary alkyl groups in aromatic compounds, including phenols, such as 17β-estradiol [16, 17]. Although this method has been improved over the years the alkylation of primary alkyl groups is still a huge challenge [17]. Most often this approach yields a mixture of rearrangement and polyalkylation products. Moreover, often harsh acidic or basic reaction conditions are required rendering the usefulness of the direct mono-alkylation of aromatics limited [18, 19]. The introduction of primary alkyl groups is therefore instead performed using multistep protocols [20-30]. These aforementioned drawbacks, as well as the challenge of achieving high regioselectivity, also apply to the synthesis of mono-alkylated estrogenic steroids, such as 2-ethyl estradiol (**4a**). Towards tackling the challenge of developing a regioselective and easy synthesis of 2-ethyl estradiol, we reported the application of a highly regioselective *ortho*-formylation protocol [31-33] of estradiols and estrogens [34]. These studies also resulted in a multi-step synthesis of 2-ethyl estradiol (**4a**) [15] that required chromatographic separation of the 2-substituted salicylaldehyde **8** from its 4-substituted regioisomer **9** (Scheme 1).



Scheme 1. Regioselective *ortho*-formylation of 17β-estradiol (**2**) yielding the two regioisomeric salicylaldehydes **8** and **9**. Compound **8** was converted into 2-ethyl estradiol (**4a**).

Since 2-substituted alkylated analogs of 2-methoxyestradiol (**1**), such as **5** and **6** (Figure 1) displayed interesting inhibition of polymerization of tubulin along with cytotoxic and anti-angiogenic effects [13-15], we became interested in conducting additional structural-activity relationship studies using **1** as the lead compound. Recently Parnes and Pappo reported a convenient and highly regioselective multi-component reductive alkylation reaction of aromatic compounds [35]. The formation of the alkylation product was mediated by an in situ formed thionium ion from an aldehyde and ethanethiol under mild catalytic acidic conditions. The resulting 1-(alkylthio)alkylarenes were reduced by triethyl silane to the mono-alkylated product in good to excellent yields. In their successful method development efforts, Parnes and Pappo reported two examples using **2** in the aforementioned protocol, affording the 2-*iso*-butyl and 2-benzyl substituted products in 67% and 85% isolated yield, respectively. These results spurred our interest in the synthesis and cytotoxic evaluations of analogs of 2-methoxyestradiol (**1**), applying this Pummerer-type reaction. These studies are communicated herein.

2. Experimental

2.1. General

Under an inert atmosphere, 17β-estradiol (**1**) (0.27 g, 1.0 mmol), aldehyde (3.0 mmol), copper (II) trifluoromethanesulfonate (9.0 mg, 2.5 mol%), and 2,2,2-trifluoroethanol (3.0 mL) were combined in a round-bottom flask. Ethanethiol (0.44 mL, 6.0 mmol) was added and the reaction mixture was stirred 16 hours at 50 °C. Triethylsilane (0.48 mL, 3.0 mmol) was then added and the mixture was allowed to stir for an additional 4 hours at 50 °C. Analysis by TLC (ethyl acetate:heptane, 30:70) indicated a complete reaction. Removal of volatiles was performed *in vacuo* where the exhaust was passed through a solution over basic potassium permanganate solution to quench excess ethanethiol. The residue was purified by flash chromatography (ethyl acetate:heptane, 30:70) to afford the products (**4a-4k**).

*2.2 (8R,9S,13S,14S,17S)-2-Ethyl-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4a**)* [15]

White solid, 80% yield (0.241 g). $[\alpha]_D^{20}$ = 82.8 (c = 0.29, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.50 (s, 1H), 3.78 – 3.70 (t, *J* = 8.4 Hz, 1H), 2.87 – 2.72 (m,

2H), 2.60 (q, *J* = 7.6 Hz, 2H), 2.34 (m, 1H), 2.22 – 2.07 (m, 2H), 1.96 (m, 1H), 1.90 – 1.82 (m, 1H), 1.75 – 1.65 (m, 1H), 1.57 – 1.12 (m, 11H), 0.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.0, 135.2, 132.3, 127.0, 126.2, 115.0, 81.8, 49.8, 43.8, 43.1, 38.7, 36.6, 30.4, 29.0, 27.1, 26.2, 23.0, 22.9, 14.2, 10.9. HRMS (EI): Exact mass calculated for $C_{20}H_{28}O_2$ $[M]^+$: 300.2089, found 300.2083.

*2.3 (8R,9S,13S,14S,17S)-2-Butyl-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4b**)*[36, 37]

White solid, 73% yield (0.241 g). $[\alpha]_D^{20}$ = 81.8 (c = 0.63, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.50 (s, 1H), 4.57 (bs, 1H, OH), 3.73 (t, *J* = 8.2Hz, 1H), 2.56 (t, *J* = 8.0 Hz, 2H), 2.38-2.29 (m, 1H), 2.22-2.08 (m, 2H), 1.99-1.92 (m, 1H), 1.90-1.81 (m, 1H), 1.75-1.65 (m, 1H), 1.63-1.14 (m, 13H), 0.94 (t, *J* = 7.3 Hz, 3H) ,0.79 (s, 3H) .^{13}C NMR (101 MHz, $CDCl_3$) δ 151.4, 135.6, 132.6, 127.26, 126.0, 115.3, 82.1, 50.2, 44.1, 43.4, 39.0, 36.9, 32.5, 30.7, 29.9, 29.4, 27.4, 26.6, 23.3, 22.9, 14.2, 11.2. HRMS (EI): Exact mass calculated for $C_{22}H_{32}O_2$ $[M]^+$: 328.2402, found 328.2404.

*2.4 (8R,9S,13S,14S,17S)-13-Methyl-2-pentyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4c**)*[36]

White solid, 69% yield (0.236 g). $[\alpha]_D^{20}$ = 79.0 (c = 0.62, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.49 (s, 1H), 3.82 – 3.63 (m, 1H), 2.87 – 2.69 (m, 2H), 2.63 – 2.47 (m, 2H), 2.41 – 2.29 (m, 1H), 2.23 – 2.04 (m, 2H), 1.99 – 1.91 (m, 1H), 1.91 – 1.82 (m, 1H), 1.76 – 1.65 (m, 1H), 1.57 (s, 3H), 1.53 – 1.43 (m, 2H), 1.40 – 1.24 (m, 8H), 1.22 – 1.13 (m, 1H), 0.89 (d, *J* = 7.3 Hz, 3H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.4, 135.6, 132.6, 127.3, 126.0, 115.4, 82.1, 50.2, 44.2, 43.4, 39.1, 36.9, 32.0, 30.8, 30.1, 30.1, 29.4, 27.4, 26.6, 23.3, 22.7, 14.2, 11.2. HRMS (EI): Exact mass calculated for $C_{23}H_{34}O_2$ $[M]^+$: 342.2559, found 342.2555.

*2.5 (8R,9S,13S,14S,17S)-2-Hexyl-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4d**)*[37]

White solid in 64% yield (0.228 g). $[\alpha]_D^{20}$ = 69.8 (c = 0.86, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.49 (s, 1H), 3.91 – 3.59 (m, 1H), 2.88 – 2.69 (m, 2H), 2.64 – 2.50 (m, 2H), 2.36 – 2.25 (m, 1H), 2.24 – 2.06 (m, 2H), 2.01 – 1.92 (m, 1H), 1.90 – 1.81 (m, 1H), 1.76 – 1.65 (m, 1H), 1.63 – 1.25 (m, 15H), 1.24 – 1.13 (m, 1H),

0.89 (m, 3H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.4, 135.6, 132.6, 127.2, 126.0, 115.4, 82.1, 50.2, 44.1, 43.4, 39.1, 36.9, 31.9, 30.8, 30.3, 30.2, 29.5, 29.4, 27.4, 26.6, 23.3, 22.8, 14.3, 11.2. HRMS (EI): Exact mass calculated for $C_{24}H_{36}O_2$ $[M]^+$: 356.2715, found 356.2714.

*2.6 (8R,9S,13S,14S,17S)-2-Isobutyl-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4e**)*[35]

White solid, 69% yield (0.229 g). $[\alpha]_D^{20}$ = 75.2 (c = 0.32, $CHCl_3$), (lit. [24] $[\alpha]_D^{22.8}$ = 70.8 ($CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (s, 1H), 6.50 (s, 1H), 3.74 (m, 1H), 2.90 – 2.70 (m, 2H), 2.48 – 2.38 (d, 2H), 2.32 (m, 1H), 2.14 (m, 2H), 1.99 – 1.82 (m, 3H), 1.75 – 1.65 (m, 1H), 1.55 – 1.13 (m, 8H), 0.94 (d, 6H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.6, 135.6, 132.4, 128.3, 124.8, 115.3, 82.1, 50.2, 44.1, 43.41, 39.4, 39.1, 36.9, 30.8, 29.4, 29.26, 27.4, 26.6, 23.3, 22.8, 22.7, 11.2. HRMS (EI): Exact mass calculated for $C_{22}H_{32}O_2$ $[M]^+$: 328.2402, found 328.2408.

*2.7 (8R,9S,13S,14S,17S)-13-Methyl-2-neopentyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4f**)*

White solid, 34% yield (0.120 g). $[\alpha]_D^{20}$ = 85.9 (c = 0.40, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.51 (s, 1H), 3.74 (t, *J* = 8.4 Hz, 1H), 2.86 – 2.76 (m, 2H), 2.47 (s, 2H), 2.30 (m, 1H), 2.14 (m, 2H), 1.95 (m, 1H), 1.86 (m, 1H), 1.70 (m, 1H), 1.54 – 1.15 (m, 8H), 0.95 (s, 9H), 0.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.0, 135.9, 132.0, 129.9, 122.9, 115.5, 82.1, 50.2, 44.1, 43.4, 43.2, 39.1, 36.9, 32.8, 30.8, 29.7, 29.3, 27.4, 26.6, 23.3, 11.3. HRMS (EI): Exact mass calculated for $C_{23}H_{34}O_2$ $[M]^+$: 342.2559, found 342.2553.

*2.8 (8R,9S,13S,14S,17S)-2-Benzyl-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4g**)*[35]

White solid, 89% yield (0.330 g). $[\alpha]_D^{20}$ = 60.5 (c = 0.94, $CHCl_3$), (lit. [24] $[\alpha]_D^{22}$ = 54.5 ($CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.16 (m, 5H), 7.05 (s, 1H), 6.52 (s, 1H), 4.02 – 3.90 (m, 2H), 3.73 (t, *J* = 8.7 Hz, 1H), 2.88 – 2.71 (m, 2H), 2.30 – 2.04 (m, 3H), 1.96 – 1.82 (m, 2H), 1.70 (m, 1H), 1.53 – 1.14 (m, 8H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.7, 140.4, 136.5, 132.8, 128.7, 128.7, 128.1, 126.4,

124.3, 115.9, 82.1, 50.2, 44.1, 43.4, 39.0, 36.8, 36.6, 30.7, 29.4, 27.4, 26.5, 23.3, 11.2. HRMS (EI): Exact mass calculated for $C_{25}H_{30}O_2$ $[M]^+$: 362.2246, found 362.2260.

*2.9 (8R,9S,13S,14S,17S)-2-(4-fluorobenzyl)-13-Methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4h**)*

Pale yellow solid, 70% yield (0.266 g). $[\alpha]_D^{20}$ = 342.7 (c = 0.33, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.16 (m, 2H), 7.02 (s, 1H), 6.99 – 6.92 (m, 2H), 6.51 (s, 1H), 3.97 – 3.85 (m, 2H), 3.76 – 3.69 (m, 1H), 2.87 – 2.73 (m, 2H), 2.29 – 2.04 (m, 3H), 1.96 – 1.82 (m, 2H), 1.76-1.66 (m, 1H), 1.74 – 1.13 (m, 8H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6 (d, J_{CF} = 245 Hz), 151.5, 136.6, 136.3 (d, J_{CF} = 3Hz), 132.9, 130.1 (d, J_{CF} = 8Hz), 128.0, 124.3, 115.8, 115.4 (d, J_{CF} = 21 Hz), 81.1, 50.2, 44.1, 43.4, 39.0 36.8, 35.6, 30.7, 29.4, 27.4, 26.5, 23.3, 11.2. HRMS (EI): Exact mass calculated for $C_{25}H_{29}FO_2$ $[M]^+$: 380.2152, found 380.2157.

*2.10 (8R,9S,13S,14S,17S)-2-(4-chlorobenzyl)-13-Methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4i**)*

Pale yellow solid, 89% yield (0.353 g). $[\alpha]_D^{20}$ = 82.6 (c = 0.84, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.02 (s, 1H), 6.50 (s, 1H), 3.99 – 3.83 (m, 2H), 3.78 – 3.65 (m, 1H), 2.91 – 2.65 (m, 2H), 2.33 – 2.21 (m, 1H), 2.21 – 2.06 (m, 2H), 1.99 – 1.81 (m, 2H), 1.75 – 1.08 (m, 9H), 0.93 – 0.85 (m, 1H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.3, 139.1, 136.5, 132.9, 131.8, 130.0, 128.6, 127.9, 123.9, 115.7, 81.9, 50.0, 43.9, 43.3, 38.9, 36.7, 35.6, 30.6, 29.2, 27.2, 26.4, 23.1, 11.1. HRMS (EI): Exact mass calculated for $C_{25}H_{29}ClO_2$ $[M]^+$: 398.1856, found 398.1861.

*2.11 (8R,9S,13S,14S,17S)-2-(4-bromobenzyl)-13-Methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4j**)*

Pale yellow solid, 84% yield (0.371 g). $[\alpha]_D^{20}$ = 74.3 (c = 1.2, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 7.01 (s, 1H), 6.49 (s, 1H), 3.96 – 3.82 (m, 2H), 3.80 – 3.68 (m, 1H), 2.90 – 2.70 (m, 2H), 2.31 – 2.20 (m, 1H), 2.21 – 2.05 (m, 2H), 1.97 – 1.81 (m, 2H), 1.76 – 1.62 (m, 1H), 1.54 – 1.11 (m, 8H), 0.89 (t, *J* = 6.6 Hz, 1H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.3, 139.7, 136.5, 132.9, 131.5, 130.4, 127.9, 123.8, 119.8, 115.7, 81.9, 50.0, 43.9, 43.3,

38.9, 36.7, 35.7, 30.6, 29.3, 27.2, 26.4, 23.1, 11.1. HRMS (EI): Exact mass calculated for $C_{25}H_{29}BrO_2$ $[M]^+$: 440.1351, found 440.1348.

*2.12 (8R,9S,13S,14S,17S)-2-(4-methoxybenzyl)-13-Methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**4k**)*

White solid, 60% yield (0.237 g). $[\alpha]_D^{20}$ = 76.8 (c = 0.573, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.12 (d, *J* = 8.70 Hz, 2H), 7.04 (s, 1H), 6.86 – 6.80 (d, *J* = 8.70 Hz, 2H), 6.52 (s, 1H), 3.95 – 3.83 (m, 2H), 3.78 (s, 3H), 3.73 (t, *J* = 8.6 Hz, 1H), 2.83 – 2.75 (m, 2H), 2.27 (m, 1H), 2.21 – 2.04 (m, 2H), 1.90 (m, 2H), 1.77-1.67 (m, 1H), 1.75 – 1.13 (m, 8H), 0.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.2, 151.7, 136.5, 132.8, 132.3, 129.7, 128.0, 124.6, 115.9, 114.2, 82.1, 55.4, 50.2, 44.1, 43.4, 39.0, 36.9, 35.8, 30.8, 29.4, 27.4, 26.5, 23.3, 11.2. HRMS (EI): Exact mass calculated for $C_{26}H_{32}O_3$ $[M]^+$: 392.2351, found 392.2328.

2.13 Cancer Cell Growth Inhibition

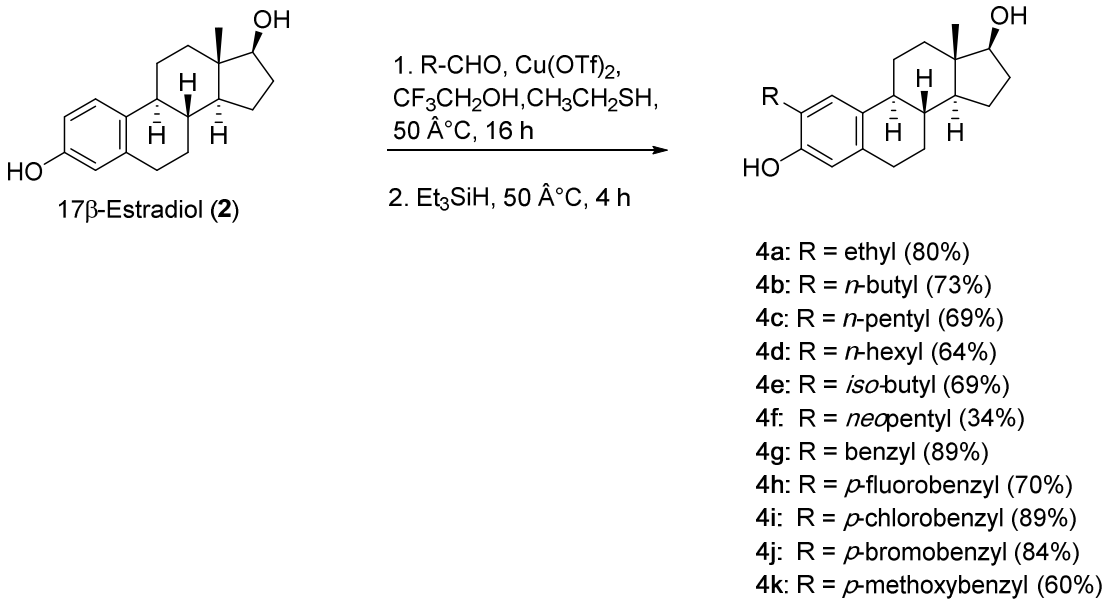
Human cervical carcinoma (HeLa) cells were seeded in 96-well plates at 15,000 cells/well in the presence of 5-fold dilutions of the compounds. After 3 days of incubation, the cells were trypsinized and counted by means of a Coulter counter (Analis, Belgium). Human dermal microvascular endothelial (HMEC-1) cells were seeded on gelatin-coated 48-well plates at 20,000 cells/well. After overnight incubation, 5-fold dilutions of the compounds were added. Three days later, the cells were trypsinized and counted. Human T-cell leukemia (CEM) cells were seeded in 96-well plates at 60,000 cells/well in the presence of the compounds, allowed to proliferate for 4 days and then counted. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to reduce cell proliferation by 50% [38].

3. Results

3.1 Chemistry

The synthesis of the analogs started with the preparation of the steroid **4a** as outlined in Scheme 2. Commercially available 17β-estradiol (**2**) was added together with acetaldehyde, copper(II) trifluoromethanesulfonate and ethanethiol and stirred in 2,2,2-trifluoroethanol at 50 °C overnight. Then triethylsilane was added, and the mixture was stirred at 50 °C for additional four hours. The desired 2-ethyl substituted

estrogen **4a** was obtained in excellent 80% isolated yield after chromatographic purification. Gratifyingly, only one regioisomer of **4a** was detected by HPLC analysis of the crude reaction mixture and by comparison with NMR data from literature [34, 35]. In the ^{1}H NMR spectrum of **4a** two singlet signals were observed at 7.06 ppm and 6.50 ppm as expected for a 2-substituted estradiol [34, 35]. The singlet at 7.06 ppm in the ^{1}H NMR spectrum is observed for H1 (Fig, 1), as determined by interpretation of the cross peaks detected for this singlet in the HSQC and HMBC NMR spectra (see Supporting information). For a 4-substituted estradiol one would expect the presence of two doublets with equal *ortho*-coupling constant integrating for one hydrogen each in the aromatic region, as reported earlier [34, 39]. The ^{13}C NMR- and the 2D NMR-spectra (COSY45, HSQC and HMBC) also supported the formation of 2-ethyl estradiol (see Supporting information). In particular the HMBC-spectrum provided additional support for the formation of 2-ethyl estradiol (**4a**) as the singlet at 7.06 ppm in the ^{1}H NMR spectrum showed correlation with signals at 22.9 ppm and 14.2 ppm in the ^{13}C NMR spectrum arising from the CH_2- and the CH_3-group in the ethyl-substituent, respectively. For the other n-alkylated aldehydes subjected to the same conditions, good to high yields in the range of 64-73% were observed for the products **4b**-**4d**, Scheme 2. Sterically more demanding aldehydes were also tested, that returned the desired 2-alkylated estrogen in 69% and 34% isolated yields of **4e** and **4f**, respectively. For 2-neopentyl estradiol (**4f**) the yield was poor, most likely due to the steric hindrance of pivaldehyde. Disappointingly, 3-methylbut-2-enal did not return any of the 2-substituted product as only the starting material **2** was recovered. The introduction of a prenyl-group in either the 2- or the 4-position of **2** would be of interest towards a biomimetic synthesis of naturally occurring terpenoid-derived estradiols [40]. When benzaldehydes were used, high (70%) to excellent yields (89%) were observed, as observed for products **4g**-**4j**. The electron-releasing *para*-methoxy benzaldehyde resulted in a 60% isolated yield of **4k**. Of note, neither 2-thiophene carboxaldehyde nor 2-furane carboxaldehyde reacted in this reaction. In both cases 17β-estradiol (**2**) was recovered unreacted. The spectral data of all known as well as new products were in accord with their assigned structures. Our attempts when using estrone or its 17-ethylene acetal with acetaldehyde gave complex reaction mixtures in both cases.



Scheme 2. Regioselective alkylation of 17β-estradiol (**2**).

3.2 Biological evaluations

The 2-alkylated analogs were submitted to human T-cell leukemia (CEM), human cervix carcinoma (HeLa) and human dermal microvascular endothelial cells (HMEC-1) for the evaluation of their anti-proliferative effects. The data are expressed as IC_{50} (50% inhibitory concentration), which is defined as the compound concentration that reduces cell proliferation by 50%, and are shown in Table 1. The reference compound 2-ME (**1**) inhibited the growth of all cell lines tested in the low micromolar range (IC_{50} between 0.4 and 1.6 µM). The most potent compound was the 2-ethyl analog **4a** with IC_{50} values of 5.6 ± 0.8 and 9.5 ± 0.9 µM towards the CEM and HeLa cell lines, respectively. None of the compounds inhibited the growth of the endothelial cell line, which may point to a tumor-selective mechanism of action. However, since they were less potent than 2-ME (**1**), no further biological studies were conducted.

Table 1. Anti-proliferative activity of 2-alkyl analogs **4a**-**4k** and 2-ME (**1**)

Compound	CEM IC_{50} (μM)[a]	HeLa IC_{50} (μM)[a]	HMEC-1 IC_{50} (μM)[a]
2-ME (**1**)	1.6 ± 0.9	0.41 ± 0.06	1.3 ± 0.5
4a	5.6 ± 0.8	9.5 ± 0.9	> 50
4b	24 ± 10	29 ± 0	> 50
4c	25 ± 8	27 ± 5	> 50
4d	18 ± 7	21 ± 4	> 50
4e	25 ± 8	24 ± 5	> 50
4f	36 ± 2	43 ± 7	> 50
4g	15 ± 0	27 ± 6	> 50
4h	35 ± 1	33 ± 11	> 50
4i	31 ± 1	33 ± 11	> 50
4j	19 ± 12	31 ± 14	> 50
4k	15 ± 3	25 ± 2	> 50

[a] Results of three experiments performed as duplicates

4. Conclusions

Structure-activity relationship (SAR) studies have shown that the endogenously formed metabolite of 17β-estradiol (**2**), namely 2-methoxyestradiol (**1**), is amendable for changes in the 2-position of the A-ring [41]. Several alkyl substituents in this position were easily introduced in **2** using a recently reported one-pot protocol [35]. The yields of the alkylated products were high to excellent, except for one example. This one-pot protocol is easy to conduct compared to the multi-step protocols previously reported [15, 36, 37, 42]. One-pot protocols offer many advantages, such as avoiding isolation, handling and chromatography of intermediates leading to time-cost benefits. The 17-acetate of 17β-estradiol (**2**), estrone and its ethylene-acetal did not react in this protocol. The 2-ethyl analog of 17β-estradiol (**2**), which displayed selective and decent cytotoxic effects, should be useful as a lead compound for further structural-activity studies where alterations are to be performed at the C17-position. Such analogs are of interest towards the development of novel analogs of 2-ME (**1**) [43].

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Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at [to be inserted by Editorial office]

REFERENCES

[1] D'Amato RJ, Lin CM, Flynn E, Folkman J, Hamel E. 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. Proc Natl Acad Sci U S A, 1994; 91: 3964-8.

[2] Perez-Perez M-J, Priego E-M, Bueno O, Martins MS, Canela M-D, Liekens S. Blocking Blood Flow to Solid Tumors by Destabilizing Tubulin: An Approach to Targeting Tumor Growth. J Med Chem, 2016; 59: 8685-8711.

[3] Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature, 1994; 368: 237-9.

[4] Peyrat J-F, Brion J-D, Alami M. Synthetic 2-methoxyestradiol derivatives: structure-activity relationships. Curr Med Chem, 2012; 19: 4142-4156.

[5] Sattler M, Quinnan LR, Pride YB, Gramlich JL, Chu SC, Even GC, Kraeft S-K, Chen LB, Salgia R. 2-Methoxyestradiol alters cell motility, migration, and adhesion. Blood, 2003; 102: 289-296.

[6] Bu S, Blaukat A, Fu X, Heldin N-E, Landstrom M. Mechanisms for 2-methoxyestradiol-induced apoptosis of prostate cancer cells. FEBS Lett, 2002; 531: 141-151.

[7] Wassberg E, Christofferson R. Angiostatic treatment of neuroblastoma. Eur J Cancer, 1997; 33: 2020-3.

[8] Sweeney C, Liu G, Yiannoutsos C, Kolesar J, Horvath D, Staab MJ, Fife K, Armstrong V, Treston A, Sidor C, Wilding G. A Phase II Multicenter, Randomized, Double-Blind, Safety Trial Assessing the Pharmacokinetics, Pharmacodynamics, and Efficacy of Oral 2-Methoxyestradiol Capsules in Hormone-Refractory Prostate Cancer. Clin Cancer Res, 2005; 11: 6625-6633.

[9] Dahut WL, Lakhani NJ, Gulley JL, Arlen PM, Kohn EC, Kotz H, McNally D, Parr A, Nguyen D, Yang SX, Steinberg SM, Venitz J, Sparreboom A, Figg WD. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. Cancer Biol Ther, 2006; 5: 22-27.
[10] James J, Murry DJ, Treston AM, Storniolo AM, Sledge GW, Sidor C, Miller KD. Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer. Invest New Drugs, 2006; 25: 41-48.
[11] Rajkumar SV, Richardson PG, Lacy MQ, Dispenzieri A, Greipp PR, Witzig TE, Schlossman R, Sidor CF, Anderson KC, Gertz MA. Novel therapy with 2-methoxyestradiol for the treatment of relapsed and plateau phase multiple myeloma. Clin Cancer Res, 2007; 13: 6162-6167.
[12] Solum EJ, Akselsen OW, Vik A, Hansen TV. Synthesis and Pharmacological Effects of the Anti-Cancer Agent 2-Methoxyestradiol. Curr Pharm Des, 2015; 21: 5453-5466.
[13] Solum EJ, Cheng J-J, Soervik IB, Paulsen RE, Vik A, Hansen TV. Synthesis and biological evaluations of new analogs of 2-methoxyestradiol: Inhibitors of tubulin and angiogenesis. Eur J Med Chem, 2014; 85: 391-398.
[14] Solum EJ, Vik A, Hansen TV. Synthesis, cytotoxic effects and tubulin polymerization inhibition of 1,4-disubstituted 1,2,3-triazole analogs of 2-methoxyestradiol. Steroids, 2014; 87: 46-53.
[15] Solum EJ, Cheng J-J, Sylte I, Vik A, Hansen TV. Synthesis, biological evaluation and molecular modeling of new analogs of the anti-cancer agent 2-methoxyestradiol: potent inhibitors of angiogenesis. RSC Adv, 2015; 5: 32497-32504.
[16] Olah GA. Friedel-Crafts Chemistry. Wiley: New York 1973.
[17] Sawama Y, Shishido Y, Kawajiri T, Goto R, Monguchi Y, Sajiki H. Iron-Catalyzed Friedel-Crafts Benzylation with Benzyl TMS Ethers at Room Temperature. Chem - Eur J, 2014; 20: 510-516.
[18] Smith MB, March J. March's Advanced Organich Chemistry: Reactions, Mechanisms, and Structure. Wiley: New York 2007.
[19] Carey FA, Sundberg RJ. Adcanced Organic Chemistry: Part A Structure and Mechanisms. Springer: New York 2007.
[20] Tsuchimoto T, Hiyama T, Fukuzawa S-i. Scandium(III) trifluoromethanesulfonate-catalyzed reductive Friedel-Crafts benzylation of aromatic compounds using arenecarbaldehydes and propane-1,3-diol. Chem Commun (Cambridge), 1996: 2345-2346.
[21] Miyai T, Onishi Y, Baba A. Indium trichloride catalyzed reductive Friedel-Crafts alkylation of aromatics using carbonyl compounds. Tetrahedron Lett, 1998; 39: 6291-6294.
[22] Miyai T, Onishi Y, Baba A. Novel reductive Friedel-Crafts alkylation of aromatics catalyzed by indium compounds: chemoselective utilization of carbonyl moieties as alkylating reagents. Tetrahedron, 1999; 55: 1017-1026.

[23] Tsuchimoto T, Tobita K, Hiyama T, Fukuzawa S-I. Scandium(III) Triflate-Catalyzed Friedel-Crafts Alkylation Reactions. J Org Chem, 1997; 62: 6997-7005.
[24] Hatakeyama T, Hashimoto T, Kondo Y, Fujiwara Y, Seike H, Takaya H, Tamada Y, Ono T, Nakamura M. Iron-Catalyzed Suzuki-Miyaura Coupling of Alkyl Halides. J Am Chem Soc, 2010; 132: 10674-10676.
[25] Gonzalez-Bobes F, Fu GC. Amino Alcohols as Ligands for Nickel-Catalyzed Suzuki Reactions of Unactivated Alkyl Halides, Including Secondary Alkyl Chlorides, with Arylboronic Acids. J Am Chem Soc, 2006; 128: 5360-5361.
[26] Molander GA, Argintaru OA, Aron I, Dreher SD. Nickel-Catalyzed Cross-Coupling of Potassium Aryl- and Heteroaryltrifluoroborates with Unactivated Alkyl Halides. Org Lett, 2010; 12: 5783-5785.
[27] Yang C-T, Zhang Z-Q, Liu Y-C, Liu L. Copper-Catalyzed Cross-Coupling Reaction of Organoboron Compounds with Primary Alkyl Halides and Pseudohalides. Angew Chem, Int Ed, 2011; 50: 3904-3907, S3904/1-S3904/108.
[28] Li C, Chen T, Li B, Xiao G, Tang W. Efficient Synthesis of Sterically Hindered Arenes Bearing Acyclic Secondary Alkyl Groups by Suzuki-Miyaura Cross-Couplings. Angew Chem, Int Ed, 2015; 54: 3792-3796.
[29] Bair JS, Schramm Y, Sergeev AG, Clot E, Eisenstein O, Hartwig JF. Linear-Selective Hydroarylation of Unactivated Terminal and Internal Olefins with Trifluoromethyl-Substituted Arenes. J Am Chem Soc, 2014; 136: 13098-13101.
[30] Robbins DW, Hartwig JF. Sterically Controlled Alkylation of Arenes through Iridium-Catalyzed C-H Borylation. Angew Chem, Int Ed, 2013; 52: 933-937.
[31] Hofsløkken NU, Skattebøl L. Convenient method for the ortho-formylation of phenols. Acta Chem Scand, 1999; 53: 258-262.
[32] Hansen TV, Skattebøl L. Ortho-formylation of phenols; Preparation of 3-bromosalicylaldehyde. Org Synth, 2005; 82: 64-68.
[33] Hansen TV, Skattebøl L. Discussion Addendum For:Ortho-Formylations Of Phenols;Preparation Of 3-Bromosalicylaldehyde. Org Synth, 2003.
[34] Akselsen ØW, Hansen TV. ortho-Formylation of estrogens. Synthesis of the anti-cancer agent 2-methoxyestradiol. Tetrahedron, 2011; 67: 7738-7742.
[35] Parnes R, Pappo D. Reductive Alkylation of Arenes by a Thiol-Based Multicomponent Reaction. Org Lett, 2015; 17: 2924-2927.
[36] Cushman M, He H-M, Katzenellenbogen JA, Lin CM, Hamel E. Synthesis, Antitubulin and Antimitotic Activity, and Cytotoxicity of Analogs of 2-Methoxyestradiol, an Endogenous Mammalian Metabolite of Estradiol That Inhibits Tubulin Polymerization by Binding to the Colchicine Binding Site. J Med Chem, 1995; 38: 2041-9.
[37] Bubert C, Leese MP, Mahon MF, Ferrandis E, Regis-Lydi S, Kasprzyk PG, Newman SP, Ho YT, Purohit A, Reed MJ, Potter BVL. 3,17-Disubstituted 2-alkylestra-1,3,5(10)-trien-3-ol derivatives: Synthesis, in vitro and in vivo anticancer activity. J Med Chem, 2007; 50: 4431-4443.
[38] Canela M-D, Perez-Perez M-J, Noppen S, Saez-Calvo G, Diaz JF, Camarasa M-J, Liekens S, Priego E-M. Novel Colchicine-Site Binders with a

Cyclohexanedione Scaffold Identified through a Ligand-Based Virtual Screening Approach. J Med Chem, 2014; 57: 3924-3938.
[39] Liu Y, Kim B, Taylor SD. Synthesis of 4-Formyl Estrone Using a Positional Protecting Group and Its Conversion to Other C-4-Substituted Estrogens. J Org Chem, 2007; 72: 8824-8830.
[40] Dewick PM. Medicinal Natural Products : A Biosynthetic Approach. John Wiley & Sons, Ltd.: Chichester 2009, p. 187-310.
[41] Verenich S, Gerk PM. Therapeutic promises of 2-Methoxyestradiol and its drug disposition challenges. Mol Pharmaceutics, 2010; 7: 2030-2039.
[42] Pert DJ, Ridley DD. Preparation of 2-isopropyloestradiol. Aust J Chem, 1988; 41: 1145-8.
[43] Tinley TL, Leal RM, Randall-Hlubek DA, Cessac JW, Wilkens LR, Rao PN, Mooberry SL. Novel 2-methoxyestradiol analogues with antitumor activity. Cancer Res, 2003; 63: 1538-1549.