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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.

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 g 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 Fig. 1.

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-ethylestradiol (**4a**). Towards tackling the challenge of developing a regioselective and easy synthesis of 2-ethylestradiol, 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-ethylestradiol (**4a**) [15] that required chromatographic separation of the 2-substituted salicylaldehyde **8** from its 4-substituted regioisomer **9** (Scheme 1).

Since 2-substituted alkylated analogs of 2-methoxyestradiol (**1**), such as **5** and **6** (Fig. 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

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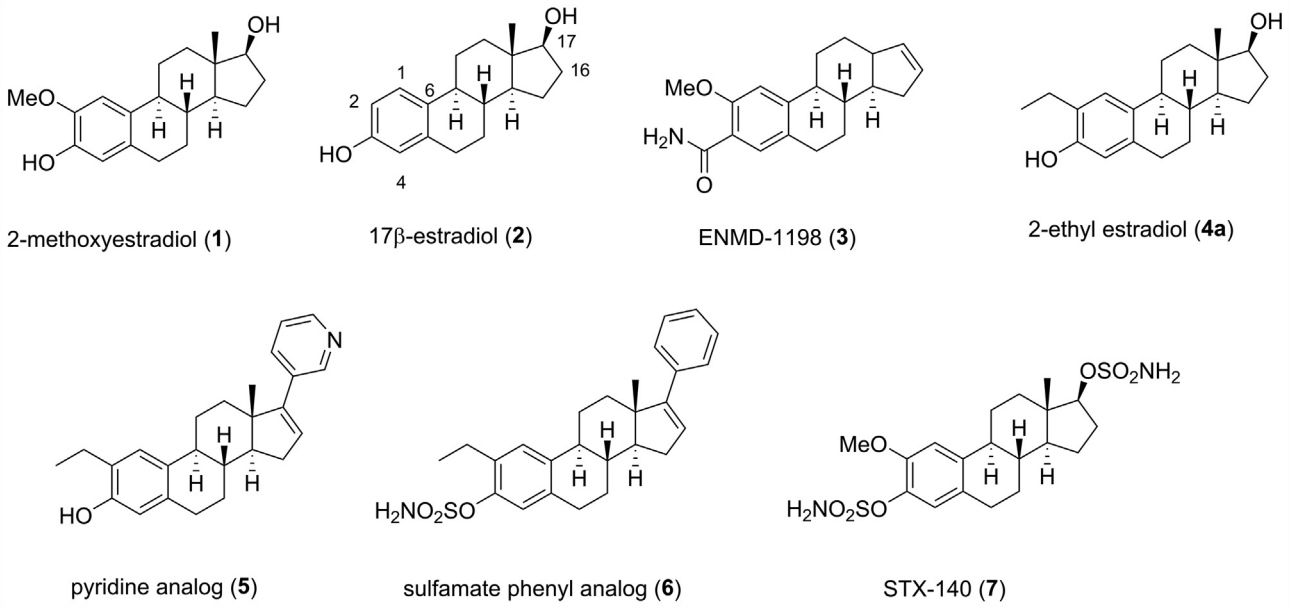
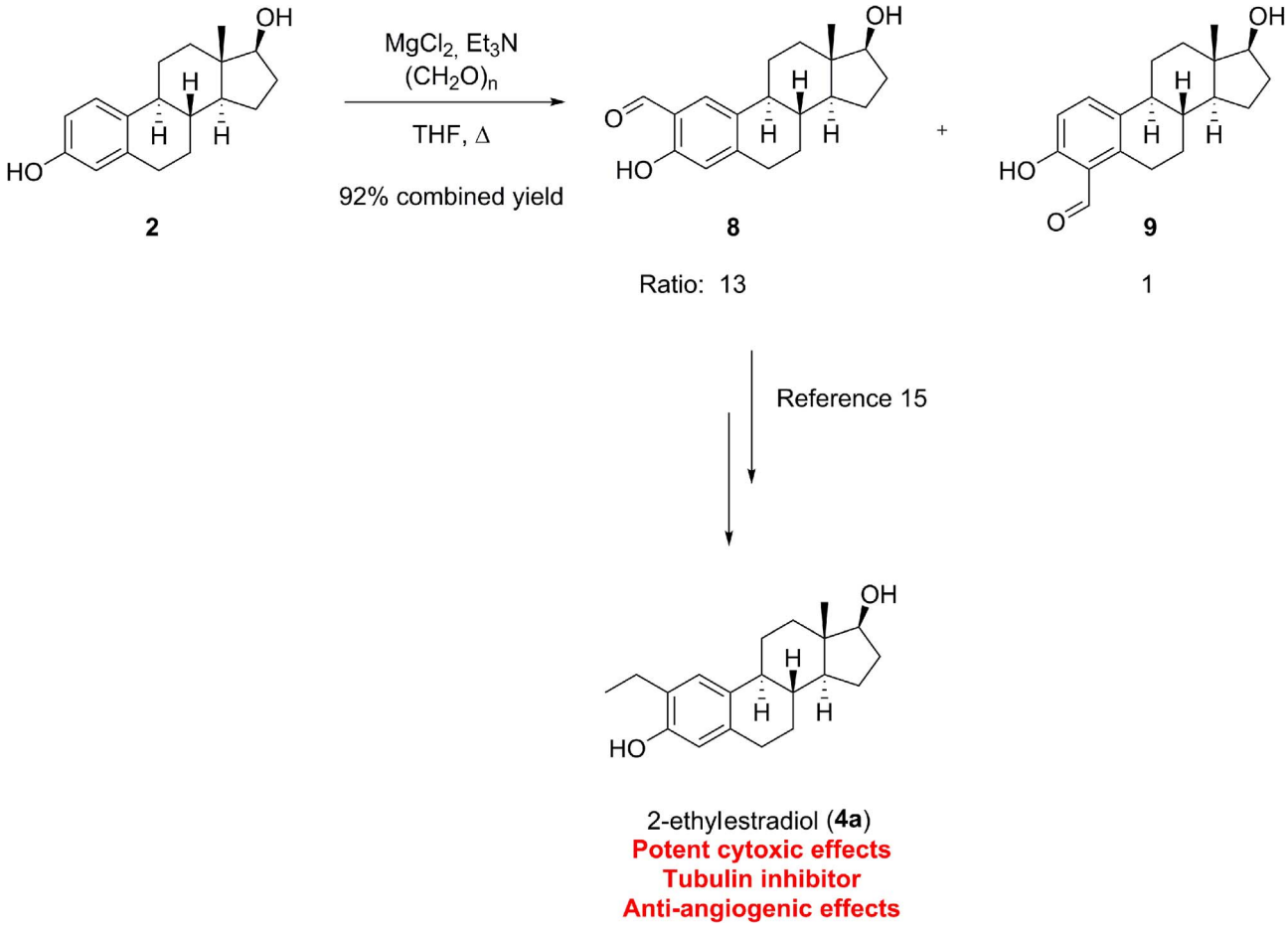


Fig. 1. Analogs of 2-ME (**1**) with anti-cancer activities.

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 triethylsilane 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-isobutyl 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



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