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Synthesis of novel estrone analogs by incorporation of thiophenols via conjugate addition to an enone side chain

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ABSTRACT

Functionalized estrogen analogs have received interest due to their unique and differing biological activity compared to their parent compounds. The synthesis of a new class of 3-methoxyestrone analogs functionalized at the C17 position possessing both alkyl and aryl substituted α,β-unsaturated ketones is described, along with their thiophenol conjugate addition products.

1. Introduction

Estrogens (estrone (**1**) (Fig. 1), estradiol, and estriol) are a set of common steroids found in both women and men that play important roles in various physiological processes [1]. Known more for their hormonal activities and contribution to the progression of estrogen-dependent breast cancer [2], estrogens and particularly their analogs have gained an increased interest due to their ability to affect other biological processes without producing the negative side effects associated with estrogen treatment.

The fact that minor modifications of the estrane structure can result in extensive changes in biological activity has led to the development of various estrane derivatives. These can be classified into two different categories. The first consists of modification of the steroid ring system itself, either through substitution of an estrane core carbon atom with a heteroatom [3], or modification of the ring systems through expansion, contraction, or additional cyclic features [4]. The second category involves addition of one or more functional groups to the estrane core structure. The most common of which tends to be the latter due to the extensive synthetic work that is necessary for the incorporation of heteroatoms into the steroid structure.

A number of research groups have been able to expand upon the known biological activity of estrogen compounds by combining them with important structural features from other natural products, creating new hybrid molecules for testing [5]. Additional estrogen hybrids or conjugates have also been synthesized to allow for receptor specific targeted delivery of a known drug candidate in an effort to increase its efficacy and minimize side effects [6]. While incorporating important chemical features of estrogens and various natural products can be effective, this is not always necessary to see evidence of modified activity.

The estradiol metabolite 2-methoxyestradiol is an example of one of these types of compounds (Fig.1) [7]. It has been reported to exhibit anticancer capabilities via microtubule disruption, anti-angiogenesis activity, and upregulation of apoptotic pathways while no longer exhibiting estrogenic characteristics [8]. However, as a result of 2-methoxyestradiol's (**2**) low bioavailability, research has continued into the synthesis of analogs concentrating on modifications at C2, C3, and C17 of the estrogen skeleton in an effort to optimize their activities (Fig.1) [9].

Even simple functionalization at the C2 position of estradiol with an adamantyl moiety provides a substrate that exhibits estrogen receptor-independent neuroprotection and vasoactive effects [10].

While modification of the A-ring of estrone (**1**) has received substantial attention, it is not the only site shown to produce interesting biological activity. Incorporation of various appendages at C17 of ring D has produced analogs that induce apoptosis in prostate cancer cell lines [11], while another is capable of simultaneous induction of autophagy and apoptosis in breast cancer cells [12] and others have shown modest cytotoxicity in human breast, lung and epidermoid carcinoma cell lines [13].

In an attempt to investigate further the biological activity associated with the C17 functionalized estrone (**1**) structure and its

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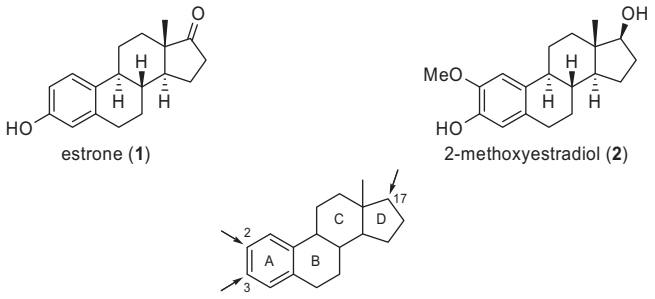


Fig. 1. Estrone (**1**), 2-methoxyestradiol (**2**), significant sites for activity.

side chain, a new set of estrogen-derived targets were identified for synthesis (Scheme 1). A key intermediate in the progression of this understanding is enone **4**. The utility of the enone functionality in organic synthesis is widely demonstrated by its use in conjugate addition [14], cycloaddition [15], organometallic [16] and many other types of synthetic reactions. The usefulness of the enone here is in the formation of β-substituted aromatic thioethers via a thiol addition to alkyl and phenyl β-substituted enones[17].

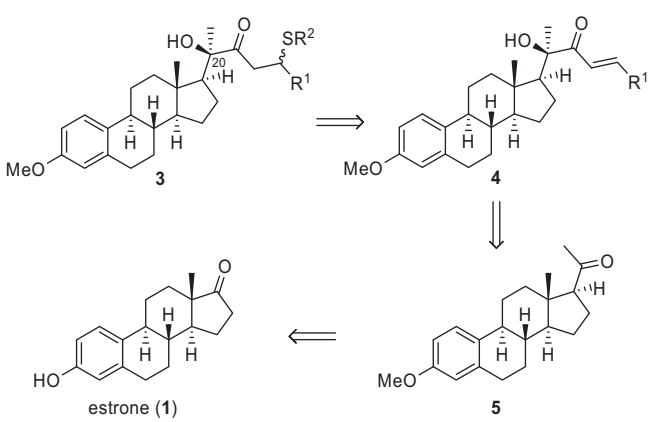
2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were acquired on a Bruker AVANCE-400 MHz NMR spectrometer, in $CDCl_3$ using TMS (δ = 0 ppm) as the internal standard for ^{1}H NMR and $CDCl_3$ (δ = 77.16 ppm) for ^{13}C NMR, with the reporting of coupling constants in Hz and the signal multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), multiplet (m), or broad (br). HRMS data was obtained using EI ionization on a ThermoFinnigan MAT 95 XL mass spectrometer. X-ray crystal structure of **8** was obtained on a Bruker APEXII diffractometer. TLC analysis was performed using pre-coated silica gel PE sheets. Products were purified via column chromatography using silica gel 40–63um (230–400 mesh), prep-plates, and reverse phase HPLC (Alltech preparative column, Econosil C18 10u, length 250 mm, ID 22 mm). All reagents and solvents were obtained from commercial suppliers and used as received. All chemical reactions requiring anhydrous conditions were performed with oven-dried glassware under an atmosphere of nitrogen.

2.2. Alkene *6*

To a stirred solution of ethyltriphenylphosphonium bromide (26.1 g, 70.2 mmol) in THF (140 mL) was added potassium *tert*-butoxide in 2 portions (7.36 g, 65.5 mmol) at room temperature for 1 h. To this solution was added estrone 3-methyl ether (6.66 g, 23.4 mmol) in a mixture of THF:DMSO (1:1, 200 mL) and then allowed to stir at 70 °C for 5 h. After cooling to room temperature, saturated NH_4Cl (150 mL) was added to quench the reaction mixture and the aqueous layer was extracted with Et_2O (3 × 200 mL) and the combined organic layers were washed with saturated NaCl (1x75 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude material was purified by silica gel column chromatography with hexanes/EtOAc (gradient; 95:5–9:1–4:1) to yield alkene **6** (6.1 g, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 8.6 Hz, 1H), 6.70 (dd, J = 8.7, 2.8 Hz, 1H), 6.62 (d, J = 2.7 Hz, 1H), 5.15 (qt, J = 7.2, 2.0 Hz, 1H), 3.77 (s, 3H), 2.96–2.78 (m, 2H), 2.47–2.17 (m, 5H), 1.97–1.88 (m, 1H), 1.79–1.67 (m, 2H), 1.69 (dt, J = 7.2, 2.0 Hz, 3H), 1.61–1.23 (m, 5H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 150.4, 138.1, 133.0, 126.4, 113.9, 113.5, 111.6, 55.3, 55.3, 44.7, 43.9, 38.5, 37.4, 31.6, 30.0, 27.7, 27.1, 24.3, 17.1, 13.3.



Scheme 1. Retrosynthetic analysis of estrone analogs.

2.3. Alcohol *7*

A solution of 9-BBN (0.5 M in THF, 52 mL, 26 mmol) was added to Wittig product **6** (2.0 g, 6.7 mmol) at room temperature. The solution was stirred until the solid starting material was dissolved, then allowed to stir at 60 °C for 18 h. After cooling to 0 °C, 50 mL of 10% NaOH and 90 mL of 30% H_2O_2 were slowly added and stirred for 1 h at 0 °C, followed by 1 h at room temperature. The aqueous layer was extracted with ethyl acetate (3 × 100 mL) and the combined organic layers were washed with saturated sodium thiosulfate (1 × 75 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude material was purified by silica gel column chromatography with hexanes/EtOAc (4:1) to yield alcohol **7** (2.0 g, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 8.7 Hz, 1H), 6.70 (dd, J = 8.5, 2.7 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 3.77–3.68 (m, 1H), 3.76 (s, 3H), 2.94–2.77 (m, 2H), 2.32–2.12 (m, 3H), 1.83–1.68 (m, 1H), 1.68–1.18 (m, 8H), 1.25 (d, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 138.1, 132.9, 126.3, 113.8, 111.5, 70.4, 58.7, 55.4, 55.3, 43.8, 42.1, 39.0, 38.5, 30.0, 27.8, 26.5, 26.0, 23.9, 23.6, 12.7.

2.4. Ketone *5*

To a stirred solution of alcohol **7** (2.0 g, 6.36 mmol) in CH_2Cl_2 (30 mL) was added 4 Å powdered molecular sieves (2.5 g), NaOAc (2.5 g, 30.5 mmol), and PCC (2.7 g, 12.7 mmol). The reaction mixture was stirred for 2 h and then Et_2O was added and the mixture was filtered over a pad of silica gel, washing with Et_2O. The filtrate was concentrated *in vacuo* to give the crude material that was purified by silica gel column chromatography with hexanes/EtOAc (4:1) to yield ketone **5** (1.9 g, 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, J = 8.6 Hz, 1H), 6.71 (dd, J = 8.6, 2.8 Hz, 1H), 6.63 (d, J = 2.7 Hz, 1H), 3.77 (s, 3H), 2.95–2.78 (m, 2H), 2.61 (dd, J = 9.2, 9.2 Hz, 1H), 2.34 (dddd, J = 12.9, 3.6, 3.6, 3.6 Hz, 1H), 2.29–2.19 (m, 2H), 2.19–2.12 (m, 1H), 2.15 (s, 3H), 1.94–1.85 (m, 1H), 1.84–1.24 (m, 8H), 0.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.6, 157.6, 138,0, 132.5, 126.3, 113.9, 111.6, 63.9, 55.8, 55.3, 44.5, 43.8, 39.1, 38.8, 31.6, 29.9, 27.8, 26.8, 24.2, 23.0, 13.5.

2.5. OTMS cyanohydrin *8*

To a stirred solution of ketone **5** (1.8 g, 5.76 mmol) in CH_2Cl_2 (12 mL) was added ZnI_2 (0.054 g, 0.17 mmol) and TMSCN (1.0 mL, 7.4 mmol). The reaction mixture was stirred for 3 h. Most of the solvent was evaporated and the remaining slurry was taken up in a water/EtOAc mixture (1:2, 90 mL) and the aqueous layer was subsequently extracted with EtOAc (2 × 50 mL), dried with Na_2-