



## Estradiol dimers as a new class of steroid sulfatase reversible inhibitors

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### ABSTRACT

A series of estradiol dimers was synthesized or selected from compounds available in our laboratory and tested for inhibition against steroid sulfatase. Dimers linked by their C17 position, compounds **7** and **8**, showed inhibitory potency similar (56% and 54% at 1  $\mu$ M) to that of our best previously reported reversible inhibitor EM-690 (62% at 1  $\mu$ M). Docking experiment seems to indicate that C17–C17 dimers bind in a similar way to EM-690 whereas C16–O3 and C16–C16 dimers bind in an upside-down position.

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Breast cancer is the most frequent cancer in women, representing about 29% of newly diagnosed cancers. For the year 2008 in Canada, it is estimated that 22,400 women will be diagnosed and that 5300 (24%) will die from this disease.<sup>1</sup> Approximately 75% of breast tumors express the estrogen receptor (ER) and are stimulated by estrogens. In these cases, it is possible to use hormone therapy (HT) as an adjuvant to classical treatments such as surgery, radiotherapy and chemotherapy.

There are two main strategies for HT (Fig. 1). In the first strategy (A), the ER is blocked using an antiestrogen (AE) or a selective estrogen receptor modulator (SERM).<sup>2</sup> In the second strategy (B), a key step of the biosynthesis of the most potent estrogen estradiol ( $E_2$ ) is blocked using an inhibitor of a steroidogenic enzyme.<sup>3</sup> This strategy has recently been successfully used in the clinic with aromatase inhibitors.<sup>4</sup> A combined approach using two compounds (AE/SERM + enzyme inhibitor) or a dual action compound is also possible.

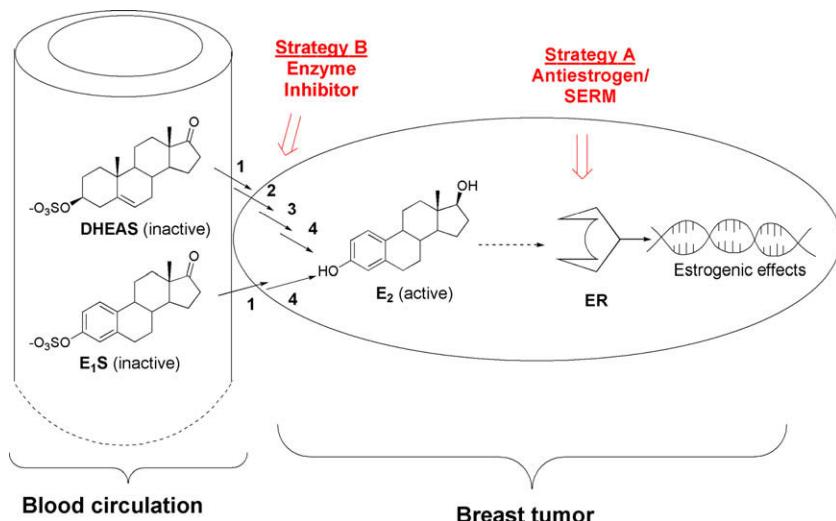
Steroidogenic enzymes other than aromatase are of therapeutic interest in breast cancer HT.<sup>5</sup> One such target enzyme is steroid sulfatase (STS), a ubiquitous enzyme which transforms inactive steroid sulfates estrone sulfate ( $E_1S$ ) and dehydroepiandrosterone sulfate (DHEAS) into active hydroxysteroids estrone ( $E_1$ ) and dehydroepiandrosterone (DHEA).<sup>6</sup> Because of their superior water solubility, steroid sulfates represent a transport form for steroids which can then be used in the local synthesis of active steroids. STS thus constitutes an important key enzyme in the control of intratumoral levels of active steroids. For this reason, there is an ongoing effort

to develop STS inhibitors, of which the first breakthrough was estrone sulfamate (EMATE),<sup>7</sup> which could not be used for breast cancer HT because of its forming estrogenic  $E_1$  upon reaction with the enzyme. The efforts since have mainly been concentrated on irreversible arylsulfamate inhibitors.<sup>8</sup>

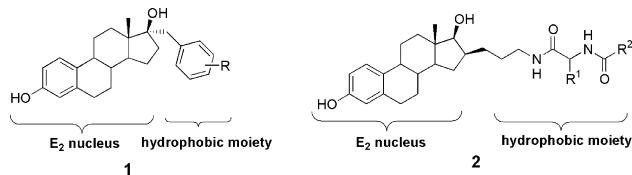
Our laboratory has developed the first reversible STS inhibitors (Fig. 2) based on  $E_2$  steroid nucleus substituted in either position 17 $\alpha$  (1)<sup>9</sup> or 16 $\beta$  (2).<sup>10</sup> The structure–activity relationship (SAR) study of these inhibitors revealed the presence of a hydrophobic pocket in the enzyme neighboring the steroid D-cycle. A few years later, the 3D structure of STS determined by X-ray crystallography showed the presence of a hydrophobic substrate entrance tunnel near its transmembrane domain (Fig. 3A).<sup>11</sup> Furthermore, preliminary kinetic studies have shown that our 17 $\alpha$ -substituted inhibitors have a non-competitive or mixed inhibition mode, which is suggestive of an allosteric binding site.<sup>12</sup> We are hypothesizing that the tunnel observed in the crystallographic structure is the hydrophobic pocket detected in our SAR study and is, in addition, the allosteric binding site detected during the kinetic studies. Thus we have designed and synthesized different dimers of  $E_2$  with which we hope to bind both active and allosteric sites to obtain a higher affinity to STS in a similar fashion to hybrid or bisubstrate inhibitors (Fig. 3B).

Different  $E_2$  dimers with a short linker were selected for synthesis based on preliminary docking experiments. Additional dimers that were already available in our laboratory and satisfied our criteria were also added in the screening test. The dimers are designated by their attachment atoms and positions, as examples C16–O3 for dimers linked from the 16-position of one  $E_2$  subunit to the 3-position of the second subunit (Fig. 4).

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**Figure 1.** The two main strategies (A and B) for breast cancer HT. The steroidogenic enzymes are steroid sulfatase (1), 3 $\beta$ -hydroxysteroid dehydrogenases (2), aromatase (3), and 17 $\beta$ -hydroxysteroid dehydrogenases (4).

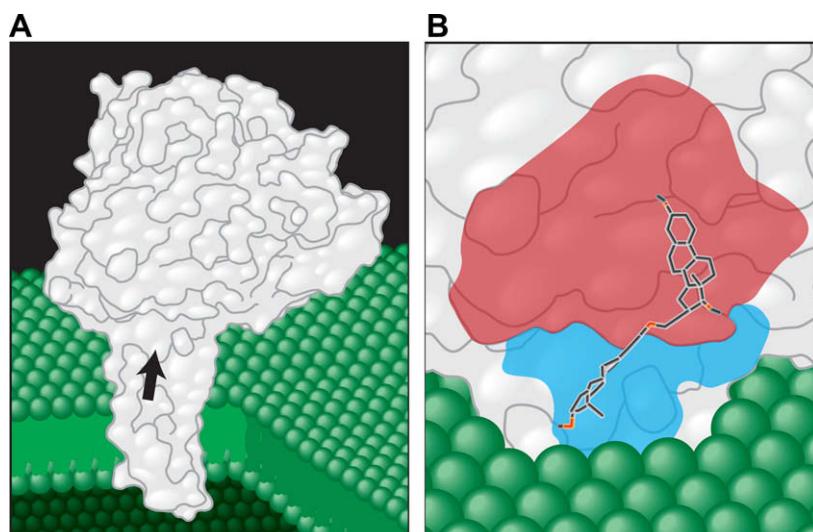


**Figure 2.** Steroidal STS inhibitors developed in our laboratory.

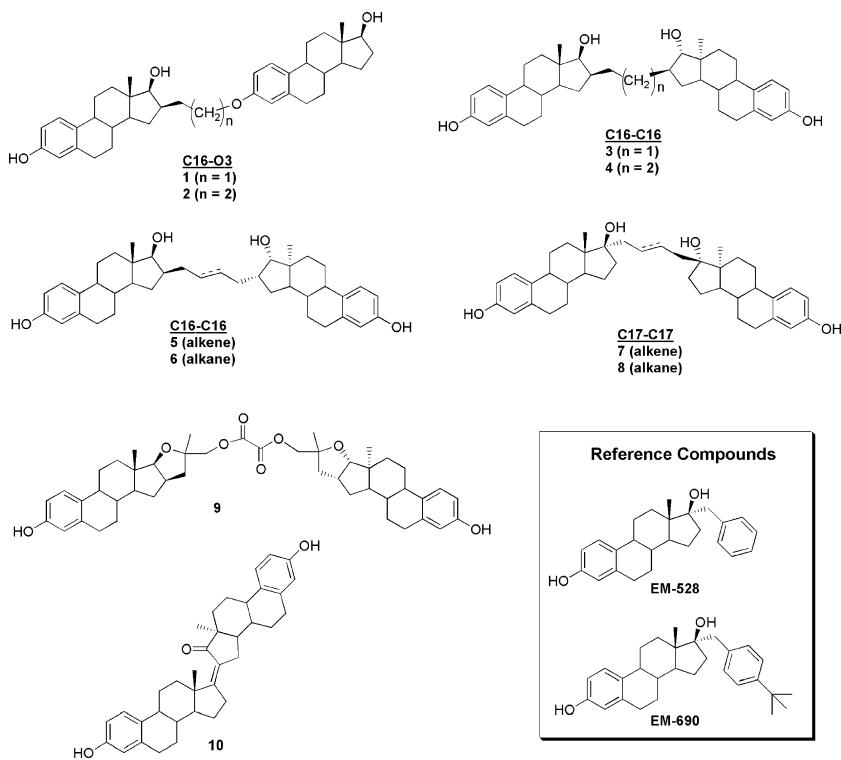
The C16–O3 dimers **1** and **2** were synthesized by O-alkylation of the phenolate group of E<sub>1</sub> with 3-O-THP-17 $\beta$ -O-TBDMS-16 $\beta$ -alkylbromide-E<sub>2</sub> followed by a reduction of the C17-ketone of E<sub>1</sub> subunit with LiAlH<sub>4</sub> and deprotection. C16–C16 dimers **3** and **4** were obtained by alkylation in C16 of 3-O-TBDMS-E<sub>1</sub> using LiHMDS as base and 3,17-diTBDMS-O-16 $\beta$ -alkylbromide-E<sub>2</sub> as nucleophile, a reduction of the C17-ketone of E<sub>1</sub> subunit and deprotection. The C16–C16 and C17–C17 dimers **5**–**8** were synthesized using a metathesis reaction as a key step. As illustrated for **7** and **8** (Fig. 5), 3-O-TBDMS-17 $\alpha$ -allyl-E<sub>2</sub><sup>13</sup> was treated with a

Grubbs's catalysis and the resulting dimer was then deprotected using tetrabutylammonium fluoride to obtain **7**.<sup>14</sup> Compound **7** was submitted to catalytic hydrogenation using palladium over charcoal and hydrogen to afford **8**.<sup>15</sup>

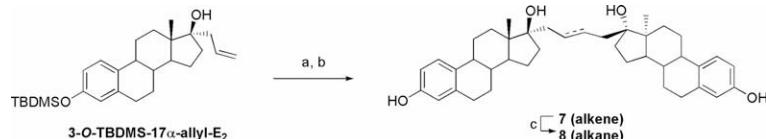
The E<sub>2</sub> dimers were tested for STS inhibition in the transformation of <sup>3</sup>H-E<sub>1</sub>S into <sup>3</sup>H-E<sub>1</sub> using homogenized HEK-293 cells over-expressing STS as the source of enzyme using a previously described procedure.<sup>16</sup> The reaction was stopped by the addition of xylene which was also used to extract the formed <sup>3</sup>H-E<sub>1</sub> from the aqueous reaction media. The results were measured by scintillation of an aliquot of both organic (<sup>3</sup>H-E<sub>1</sub>) and aqueous (<sup>3</sup>H-E<sub>1</sub>S) phases and are reported as percentage of inhibition (Fig. 6). EM-528 and EM-690,<sup>9</sup> both among our best previously reported E<sub>2</sub>-nucleus reversible phenolic inhibitors, were used as reference compounds. Their substituent at position 17 $\alpha$  is supposed at less partially to occupy the hydrophobic tunnel targeted by new dimer inhibitors. As can be seen in the inhibition assay, no inhibitor was significantly active at 0.01  $\mu$ M. However, at 0.1  $\mu$ M, compounds **7** and **8** showed 42% and 30% inhibition respectively, compared with 15% for EM-528 and 39% for EM-690. At 1  $\mu$ M, compounds **7**, **8** and



**Figure 3.** (A) Representation of STS and its substrate entrance tunnel (arrow). (B) Representation of a steroid dimer bound inside STS. The active site is shown in red, the putative access tunnel in blue and the endoplasmic reticulum membrane in green. Adapted from Figure 6a in Ref. 11.



**Figure 4.** The different E<sub>2</sub> dimers selected for testing as STS inhibitors.



**Figure 5.** Synthesis of compounds **7** and **8**. Reagents and conditions: (a) Grubbs's catalyst second generation, CH<sub>2</sub>Cl<sub>2</sub>, 40 °C, 16 h; (b) TBAF, THF, rt, 1 h; (c) Pd/C, H<sub>2</sub>, EtOAc, EtOH, rt, 16 h.

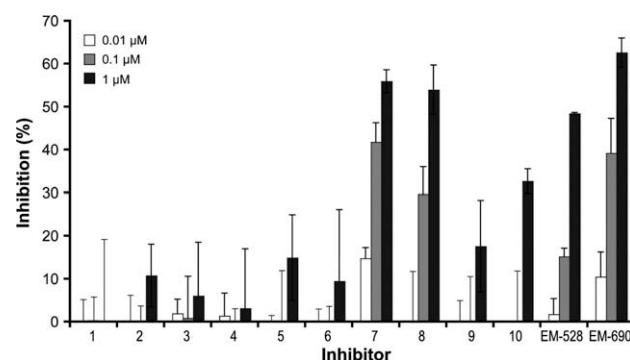
**10** showed 56%, 54% and 32% inhibition, respectively, while EM-528 and EM-690 inhibited 48% and 62% of the transformation of E<sub>1</sub>S into E<sub>1</sub>. Clearly, among the differently bound compounds, only the C17-C17 linked dimers have shown significant inhibitory activity against STS. Compound **7**, the best of this series, shows an inhibitory potency similar to that of our best reported phenolic reversible inhibitor EM-690.<sup>9</sup>

Our preliminary docking studies led us to expect a significantly higher inhibition for the C16-O3 dimer **1** compared with either C16-C16 (compounds **3–6**) or C17-C17 (compounds **7** and **8**) dimers. We therefore decided to compare the lowest energy docked conformation of EM-690 and dimers **7** and **1** using the Autodock 4 docking software.<sup>17</sup>

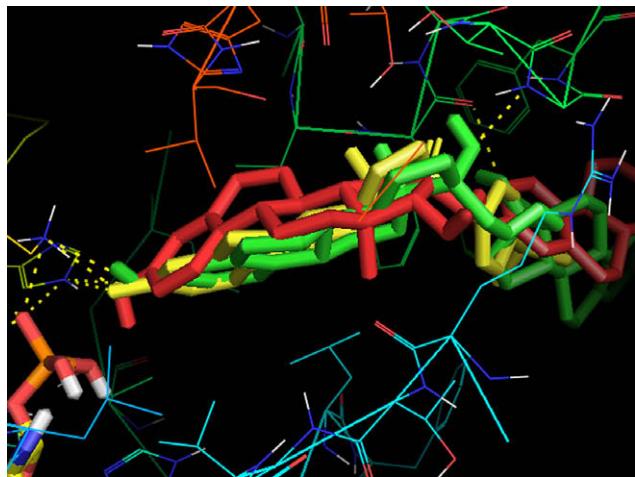
Docking experiments were performed 50 times for each compound using the Lamarckian genetic algorithm. Figure 7 shows the superposed best conformations for the three compounds. As can be seen in this figure, the active site binding moiety of **7** and EM-690 bind in a quite similar manner, whereas that of **1** binds upside-down, with its C18 methyl group pointing in the opposite direction to that of EM-690. Considering that the available structure of STS is an apoenzyme structure with a low resolution of 2.5 Å, this could explain why the energy evaluation obtained with scoring cannot correctly distinguish the two binding modes. In addition, very few features of substrate binding have been described until now for this enzyme. The only complex structure

mentioned in literature is with E<sub>1</sub>,<sup>18</sup> which is described as having mixed inhibition behavior, and shows a weak electron density at the enzyme active site, indicative of high thermal motion. However, STS seems to possess an estrogen recognition motif (leu 103, val 486), which could explain why it does not tolerate the upside-down active site binding of **1**.

In conclusion, a new type of reversible STS inhibitors, steroid dimers, have been synthesized, characterized and tested. Among the



**Figure 6.** Inhibition assay against STS in the transformation of <sup>3</sup>H-E<sub>1</sub>S into <sup>3</sup>H-E<sub>1</sub>. Results are reported as % inhibition at three inhibitor concentrations (0.01, 0.1, and 1 μM).



**Figure 7.** Superposition of the best docking conformations of EM-690 (yellow), dimer 7 (green), and dimer 1 (red).

tested compounds, only C17–C17 dimers have shown significant inhibitory activity, with compound **7** having similar activity to our previously reported best phenolic inhibitor EM-690. Our docking experiment seems to indicate that C17–C17 dimers bind in a similar way to EM-690 whereas C16–O3 and C16–C16 dimers bind in an upside-down position. Following these conclusions, the synthesis of a new series of C17–C17 dimers with various linker chain lengths, hydroxylated or deoxygenated at C3 position, is under way.

## Acknowledgments

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14. Compound **7**: IR  $\nu$  (KBr pellet) 3382 (OH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz)  $\delta$  (DMSO- $d_6$ ) 0.80 (s, 6H,  $\text{CH}_3$ -18), 1.20–2.30 (residual CH and  $\text{CH}_2$ ), 2.70 (m, 4H,  $\text{CH}_2$ -6) 4.03 (s, 2H, 17-OH), 5.60 (t, 2H,  $J$  = 2.7 Hz,  $\text{CH}=\text{CH}$ ), 6.43 (d, 2H,  $J$  = 2.1 Hz,  $\text{CH}$ -4), 6.50 (dd, 2H,  $J_1$  = 2.2 Hz and  $J_2$  = 8.3 Hz,  $\text{CH}$ -2), 7.04 (d, 2H,  $J$  = 8.5 Hz,  $\text{CH}$ -1), 9.01 (s, 2H, 3-OH) ppm;  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  (DMSO- $d_6$ ) in ppm 14.65 (2 $\times$ ), 23.21 (2 $\times$ ), 26.12 (2 $\times$ ), 27.17 (2 $\times$ ), 29.24 (2 $\times$ ), 31.16 (2 $\times$ ), 33.16 (2 $\times$ ), 39.56 (2 $\times$ ), 40.62 (2 $\times$ ), 43.29 (2 $\times$ ), 46.24 (2 $\times$ ), 49.05 (2 $\times$ ), 81.82 (2 $\times$ ), 112.70 (2 $\times$ ), 114.92 (2 $\times$ ), 126.04 (2 $\times$ ), 129.84 (2 $\times$ ), 130.51 (2 $\times$ ), 137.18 (2 $\times$ ), 154.90 (2 $\times$ ) ppm; LRMS 595.5 m/z for  $[\text{M}-\text{H}]^-$  ( $\text{C}_{40}\text{H}_{51}\text{O}_4$ ).
15. Compound **8**: IR  $\nu$  (KBr pellet) 3413 (OH)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz)  $\delta$  (DMSO- $d_6$ ) 0.79 (s, 6H,  $\text{CH}_3$ -18), 1.20–2.30 (residual CH and  $\text{CH}_2$ ), 2.69 (m, 4H,  $\text{CH}_2$ -6) 3.84 (s, 2H, 17-OH), 6.43 (d, 2H,  $J$  = 2.2 Hz,  $\text{CH}$ -4), 6.50 (dd, 2H,  $J_1$  = 2.2 Hz and  $J_2$  = 8.3 Hz,  $\text{CH}$ -2), 7.03 (d, 2H,  $J$  = 8.5 Hz,  $\text{CH}$ -1), 9.00 (s, 2H, 3-OH) ppm;  $^{13}\text{C}$  NMR (100 MHz)  $\delta$  (DMSO- $d_6$ ) 14.65 (2 $\times$ ), 23.15 (2 $\times$ ), 24.56 (2 $\times$ ), 26.16 (2 $\times$ ), 27.21 (2 $\times$ ), 29.24 (2 $\times$ ), 31.34 (2 $\times$ ), 33.21 (2 $\times$ ), 36.79 (2 $\times$ ), 43.38 (2 $\times$ ), 46.37 (2 $\times$ ), 49.08 (2 $\times$ ), 81.75 (2 $\times$ ), 112.69 (2 $\times$ ), 114.92 (2 $\times$ ), 126.02 (2 $\times$ ), 130.52 (2 $\times$ ), 137.17 (2 $\times$ ), 154.90 (2 $\times$ ) ppm; LRMS 597.5 m/z for  $[\text{M}-\text{H}]^-$  ( $\text{C}_{40}\text{H}_{53}\text{O}_4$ ).
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