and DCM (1×), and dried to afford resins of general structure **162**. The third level was introduced on resins **162** by coupling carboxylic acid building block **156**. A stock solution of **156** (1.60 g, 6.40 mmol), PyBrOP (2.98 g, 6.40 mmol) and HOBt (865 mg, 6.40 mmol) was prepared in dry DMF (50 mL) and DIPEA (2.23 mL, 12.8 mmol) was added. To each of the 25 resins **162** was added 2.0 mL of the stock solution. The mixtures were shaken under argon for 4 h at room temperature, then filtered, washed with MeOH (1×), DMF (2×), DCM (3×), MeOH (3×) and DCM (1×), and dried under a vacuum to afford resins of general structure **163**. The completion of the coupling reaction was verified by acidic mini-cleavage and TLC analysis.

4.2.19. Removal of protective groups (library D)

Resins **163** that were prepared with Fmoc-*L*-Ser(Trt)-OH or Fmoc-*L*-Tyr(2-Cl-Trt)-OH building blocks were treated with 2.0 mL of a solution of TFA/TIS (triisopropylsilane)/DCM, 2:1:97 for 10 min at room temperature. The resins were filtered and washed with DCM (3×), MeOH (3×) and DCM (3×). This procedure was performed twice in order to completely remove the trityl or the 2-Cl-trityl protective groups. The deprotection step was completed when the solution of acidic mini-cleavage of a sampling of resins **163** (after step e) did not become yellow anymore. The THP protective group was next removed in mild acid condition. A solution of *p*-TSA (666 mg, 3.5 mmol) dissolved in *t*-BuOH (25 mL) and DCM (25 mL) was prepared and 2.0 mL of this solution was added to each of the 25 resins **163** (after step e, if necessary). The mixtures were shaken for 24 h at room temperature. They were then filtered, washed with DCM (3×), MeOH (3×) and DCM (1×), and dried under a vacuum to afford resins **164**.

4.2.20. Generation of E_2 derivatives 165 by nucleophilic cleavage

To each of the 25 resins **164** was added 2.0 mL of a solution of DEA (30%) in THF. The mixtures were shaken for 45 h at room temperature. Then 1.5 mL of a solution of DEA (30%) in THF was added to each of the 25 resins and the mixtures were shaken for an additional 20 h. The mixtures were then filtered and washed with THF (3×). The filtrates were collected in preweighed tubes and evaporated in a Speedvac apparatus. Each compound was dissolved in THF, evaporated twice and dried under a vacuum pump in order to obtain the DEA-free product. The phenol derivatives **165** were obtained in the form of a yellowish viscous oil. A random sampling was done and 2 compounds were analyzed by ^{1}H NMR to verify their structure.

16β-(N-[3'-(Acetylbutyl ester)]-phenylacetyl-L-tyrosine-L-phenylalanine-aminopropyl)-3,17β-dihydroxy-estra-1,3,5(10)-triene (**165**: R_1 = a, R_2 = e). ^{1}H-NMR (400 MHz, methanol-d_4) δ 0.79 (s, 18-CH_3), 0.85 to 2.35 (m, CH and CH_2 of steroid skeleton and alkyl chain), 1.44 (s, $(CH_3)_3CO$), 2.78 (m, 6-CH_2), 2.93 and 3.12 (2m, CHC$\underline{H}_2$Ph, CHC$\underline{H}_2$PhOH and C$\underline{H}_2$NH), 3.46 and 3.48 (2s, COC$\underline{H}_2$Ph), 3.51 (s, C$\underline{H}_2$COO*t*Bu), 3.75 (under THF peaks, 17α-CH), 4.52 (m, C$\underline{H}$CH_2Ph and C$\underline{H}$CH_2PhOH), 6.49 (d, J = 2.4 Hz, 4-CH), 6.55 (dd, J_1 = 8.4 Hz, J_2 = 2.6 Hz, 2-CH), 6.65 (d, J = 8.5 Hz, 3'-CH and 5'-CH of 4-hydroxyphenyl),

6.94 (d, J = 8.5 Hz, 2'-CH and 6'-CH of 4-hydroxyphenyl), 7.01 (d, J = 7.5 Hz, 1-CH), 7.14 (m, 9 × CH of phenyl).

16β-(N-[3'-(Acetylbutyl ester)]-phenylacetyl-L-serine-L-aspartic acid-aminopropyl)-3,17β-dihydroxy-estra-1,3,5(10)-triene (**165**: R_1 = c, R_2 = d). ^{1}H-NMR (400 MHz, methanol-d_4) δ 0.80 (s, 18-CH_3), 0.85 to 2.35 (m, CH and CH_2 of steroid skeleton and alkyl chain), 1.45 (s, $(CH_3)_3CO$), 2.78 (m, 6-CH_2 and C$\underline{H}_2$COOH), 3.15 (m, C$\underline{H}_2$NH), 3.54 (s, COC$\underline{H}_2$Ph), 3.62 (s, C$\underline{H}_2$COO*t*Bu), 3.75 (under THF peaks, 17α-CH), 3.75 and 3.86 (under THF peaks and dd, J_1 = 10.6 Hz, J_2 = 5.5 Hz, C$\underline{H}_2$OH), 4.22 (t, J = 6.0 Hz, C$\underline{H}$CH_2OH), 4.75 (m, C$\underline{H}$CH_2COOH), 6.49 (d, J = 2.3 Hz, 4-CH), 6.55 (dd, J_1 = 8.4 Hz, J_2 = 2.6 Hz, 2-CH), 7.09 (d, J = 8.4 Hz, 1-CH), 7.24 (m, 4 × CH of phenyl).

4.2.21. Generation of E_2 derivatives (library D) by an acid treatment

In order to remove the *tert*-butyl ester protective group, each compound **165** was put in a tube and treated for 3 h at room temperature with 2.0 mL of HCl (4M in dioxane). The reaction mixtures were then evaporated to dryness under reduced pressure and dried overnight under a vacuum pump. The crude products were dissolved in MeOH and preadsorbed on C-18 silica gel. Purification by a C-18 silica gel column (Honeywell Burdick & Jackson, solid phase systems C18 columns, size 2000 mg/8 mL (product # 9009) distributed by VWR) afforded carboxylic acid, methyl ester or a mixture of carboxylic acid and methyl ester derivatives **166–190** (2–31 mg, 2–33% overall yield from **158**). LRMS and notes on ^{1}H-NMR analysis of each library members are presented in Table 4. ^{1}H-NMR of a random sampling of four members is described as examples.

166 *(methyl ester)*. ^{1}H-NMR (400 MHz, methanol-d_4) δ 0.79 (s, 18-CH_3), 0.95 to 2.35 (m, CH and CH_2 of steroid skeleton and alkyl chain), 2.79 (m, 6-CH_2), 2.90 and 3.10 (2m, 2 × CHC$\underline{H}_2$Ph and C$\underline{H}_2$NH), 3.45 and 3.46 (2s, COCH_2Ph), 3.61 (s, C$\underline{H}_2$COOCH_3), 3.67 (s, COOCH_3), 3.71 (d, J = 9.8 Hz, 17α-CH), 4.53 (t, J = 7.3 Hz, COC$\underline{H}$NH), 4.60 (m, COC$\underline{H}$NH), 6.48 (d, J = 2.5 Hz, 4-CH), 6.55 (dd, J_1 = 8.4 Hz, J_2 = 2.5 Hz, 2-CH), 7.01 (d, J = 7.5 Hz, 1-CH), 7.05 to 7.30 (m, 14 × CH of phenyl).

180 *(methyl ester)*. ^{1}H-NMR (400 MHz, methanol-d_4) δ 0.80 (s, 18-CH_3), 1.00 to 2.35 (m, CH and CH_2 of steroid skeleton and alkyl chain), 2.75 (m, 6-CH_2), 2.80, 3.00 and 3.14 (3m, CHC$\underline{H}_2$COOH, C$\underline{H}_2$PhOH) and C$\underline{H}_2$NH), 3.54 (m, COC$\underline{H}_2$Ph), 3.63 and 3.66 (2s, C$\underline{H}_2$COOCH_3), 3.68 (s, COOCH_3), 3.71 (d, J = 9.7 Hz, 17α-CH), 4.48 (m, C$\underline{H}$CH_2PhOH), 4.64 (m, C$\underline{H}$CH_2COOH), 6.49 (d, J = 2.5 Hz, 4-CH), 6.55 (dd, J_1 = 8.3 Hz, J_2 = 2.6 Hz, 2-CH), 6.68 (m, 2 × CH of PhOH), 7.00 to 7.25 (m, 1-CH, 2 × CH of PhOH and 4 × CH of Ph).

184 *(carboxylic acid)*. ^{1}H-NMR (400 MHz, methanol-d_4) δ 0.79 (s, 18-CH_3), 0.95 to 2.35 (m, CH and CH_2 of steroid skeleton and alkyl chain), 2.78 (m, 6-CH_2), 3.20 (m, C$\underline{H}_2$NH), 3.60 and 3.63 (2s, COC$\underline{H}_2$Ph), 3.65 and 3.69 (2s, C$\underline{H}_2$COOH), 3.71 (d, J = 9.9 Hz, 17α-CH), 3.79 and 3.89 (2m, 2 × C$\underline{H}_2$OH), 4.39 and

4.45 (2m, 2 × C<u>H</u>CH$_2$OH), 6.49 (d, J = 2.4 Hz, 4-CH), 6.55 (dd, J_1 = 8.4 Hz, J_2 = 2.5 Hz, 2-CH), 7.09 (d, J = 8.4 Hz, 1-CH), 7.24 (m, 4 × CH of Ph).

185 *(mix of methyl ester and carboxylic acid)*. ^{1}H-NMR (400 MHz, methanol-d$_4$) δ 0.80 (s, 18-CH$_3$), 0.90 to 2.35 (m, CH and CH$_2$ of steroid skeleton and alkyl chain), 2.78 (m, 6-CH$_2$), 2.90 and 3.05 (2m, C<u>H</u>$_2$PhOH), 3.17 (m, C<u>H</u>$_2$NH), 3.52 and 3.53 (2s, COC<u>H</u>$_2$Ph), 3.58, 3.63 and 3.65 (3s, C<u>H</u>$_2$COOX, × = H or CH$_3$), 3.68 (s, COOCH$_3$), 3.72 and 3.79 (2m, 17α-CH and C<u>H</u>$_2$OH), 4.33 (m, C<u>H</u>CH$_2$PhOH), 4.60 (m, C<u>H</u>CH$_2$OH), 6.49 (s, 4-CH), 6.55 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 2-CH), 6.68 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 2 × CH of PhOH), 7.00 to 7.35 (m, 1-CH, 2 × CH of PhOH and 4 × CH of Ph).

4.3. Inhibition of 17β-HSD1 in homogenated cells (enzymatic assay)

The enzymatic assays on 17β-HSD1 were performed as previously described [15,41]. HEK-293 cells transfected with 17β-HSD1 cDNA fragment were briefly sonicated in 50 mM sodium phosphate buffer (pH 7.4) containing 20% glycerol and 1mM EDTA to obtain cellular fragmentation. The cytosol fraction containing the enzyme was isolated as the supernatant after centrifugation (100,000g, 5 min, 4 °C). The enzymatic reaction was performed at 37 °C for 2 h in 1 mL of a solution which included 870 µL of 50 mM sodium phosphate buffer (pH 7.4, 20% glycerol and 1 mM EDTA), 100 µL of 1 mM NADH in phosphate buffer, 10 µL of 10 µM [^{14}C]-estrone in ethanol (54 mCi / mmol, American Radiolabeled Chemicals Inc., St-Louis, MO, USA), 10 µL of indicated inhibitor dissolved in ethanol and 10 µL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in duplicate at a final concentration of 1µM. Radiolabeled steroids were then extracted twice from the reaction mixture by 1 mL of diethyl ether. The organic phases were pooled and evaporated to dryness with nitrogen. Residues were dissolved in 50 µL of DCM, applied on silica gel 60 F_{254} thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with a mixture of toluene/acetone (4:1). Substrate ([^{14}C]-E_1) and metabolite ([^{14}C]-E_2) were identified by comparison with reference steroids and quantified using the Storm 860 system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation of [^{14}C]-E_1 into [^{14}C]-E_2 was calculated as follows: % transformation = 100 × ([^{14}C]-E_2/([^{14}C]-E_2 + [^{14}C]-E_1)), and subsequently, % inhibition = 100 × [(% transformation without inhibitor - % transformation with inhibitor)/% transformation without inhibitor].

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References

1. Peltoketo, H.; Isomaa, V.; Maentausta, O.; Vihko, R. Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett.* **1988**, *239*, 73–77.
2. Moeller, G.; Adamski, J. Integrated view on 17beta-hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* **2009**, *301*, 7–19.
3. Luu-The, V. Analysis and characteristics of multiple types of human 17β-hydroxysteroid dehydrogenase. *J. Steroid Biochem. Mol. Biol.* **2001**, *76*, 143–151.
4. Penning, T.M. 17β-hydroxysteroid dehydrogenase: inhibitors and inhibitor design. *Endocr. Relat. Cancer* **1996**, *3*, 41–56.
5. Geisler, J.; Berntsen, H.; Lonning, P.E. A novel HPLC-RIA method for the simultaneous detection of estrone, estradiol, and estrone sulphate levels in breast cancer tissue. *J. Steroid Biochem. Mol. Biol.* **2000**, *72*, 259–264.
6. Miyoshi, Y.; Ando, A.; Shiba, E.; Taguchi, T.; Tamaki, Y.; Noguchi, S. Involvement of up-regulation of 17beta-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers. *Int. J. Cancer* **2001**, *94*, 685–689.
7. Langer, L.J.; Engel, L.L. Human placental estradiol-17 beta dehydrogenase. I. Concentration, characterization and assay. *J. Biol. Chem.* **1958**, *233*, 583–588.
8. Ryan, K.J.; Engel, L.L. The interconversion of estrone and estradiol by human tissue slices. *Endocrinology* **1953**, *52*, 287–291.
9. Poirier, D. Recent advances in development of inhibitors of 17β-hydroxysteroid dehydrogenases. *Anti-Cancer Agents Med. Chem*. **2009**, *9*, 642–660.
10. Day, J.M.; Tutill, H.J.; Purohit, A.; Reed, M.J. Design and validation of specific inhibitors of 17β-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. *Endocr-Relat. Cancer* **2008**, *15*, 665–692.
11. Brozic, P.; Lanisnik Risner, T.; Gobec, S. Inhibitors of 17β-hydroxysteroid dehydrogenase type 1. *Curr. Med. Chem.* **2008**, *15*, 137–150.
12. Poirier, D. New cancer drugs targeting the biosynthesis of estrogens and androgens. *Drug Dev. Res.* **2008**, *69*, 304–318.
13. Poirier, D. Inhibitors of 17β-hydroxysteroid dehydrogenases. *Curr. Med. Chem.* **2003**, *10*, 1241–1253.
14. Smith, H.J.; Nicholls, P.J.; Simons, C.; Le Lain, R. Inhibitors of steroidogenesis as agents for the treatment of hormone-dependant cancers. *Exp. Opin. Ther. Patents* **2001**, *11*, 789–824.
15. Poirier, D.; Boivin, R.P.; Tremblay, M.R.; Bérubé, M.; Lin, S.X. Estradiol−adenosine hybrid compounds designed to inhibit type 1 17β-hydroxysteroid dehydrogenase. *J. Med. Chem.* **2005**, *48*, 8134–8147.
16. Poirier, D.; Boivin, R.P.; Bérubé, M.; Lin, S.X. Synthesis of a first estradiol-adenosine hybrid compound. *Synth. Commun*. **2003**, *33*, 3183–3191.

17. Qiu, W.; Campbell, R.L.; Gangloff, A.; Dupuis, P.; Boivin, R.P.; Tremblay, M.R.; Poirier, D.; Lin, S.X. A concerted, rational design of type 1 17β-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity. *FASEB J.* **2002**, *16*, 1829–1831.
18. Bérubé, M.; Poirier, D. Synthesis of simplified hybrid inhibitors of type 1 17β-hydroxysteroid dehydrogenase via cross-metathesis and Sonogashira coupling reactions. *Org. Lett.* **2004**, *6*, 3127–3130.
19. Bérubé, M. Poirier, D. Design, chemical synthesis, and *in vitro* biological evaluation of simplified estradiol–adenosine hybrids as inhibitors of 17β-hydroxysteroid dehydrogenase type 1. *Can. J. Chem*. **2009**, *87*, 1180–1199.
20. Fournier, D.; Poirier, D.; Mazumdar, M.; Lin, S.X. Design and synthesis of bisubstrate inhibitors of type 1 17β-hydroxysteroid dehydrogenase: Overview and perspectives. *Eur. J. Med. Chem.* **2008**, *43*, 2298–2306.
21. Mazza, C.; Breton, R.; Housset, D.; Fontecilla-Camps, J.C. Unusual charge stabilization of $NADP^+$ in 17β-hydroxysteroid dehydrogenase. *J. Biol. Chem.* **1998**, *273*, 8145–8152.
22. Lawrence, H.R.; Vicker, N.; Allan, G.M.; Smith, A.; Mahon, M.F.; Tutill, H.J.; Purohit, A.; Reed, M.J.; Potter, B.V.L. Novel and potent 17β-hydroxysteroid dehydrogenase type 1 inhibitors. *J. Med. Chem.* **2005**, *48*, 2759–2762.
23. Allan, G.M.; Lawrence, H.R.; Cornet, J.; Bubert, C.; Fischer, D.S.; Vicker, N.; Smith, A.; Tutill, H.J.; Purohit, A.; Day, J.M.; Mahon, M.F.; Reed, M.J.; Potter, B.V.L. Modification of estrone at the 6, 16, and 17 positions: novel potent inhibitors of 17 β -hydroxysteroid dehydrogenase type 1. *J. Med. Chem.* **2006**, *49*, 1325–1345.
24. Vicker, N.; Lawrence, H.R.; Allan, G.M.; Bubert, C.; Smith, A.; Tutill, H.J.; Purohit, A.; Day, M.J.; Mahon, M.F.; Reed, M.J.; Potter, B.V.L. Focused libraries of 16-substituted estrone derivatives and modified e-ring steroids: inhibitors of 17β-hydroxysteroid dehydrogenase type 1. *Chem. Med. Chem.* **2006**, *1*, 464–481.
25. Laplante, Y.; Cadot, C.; Fournier, M. A.; Poirier, D. Estradiol and estrone C-16 derivatives as inhibitors of type 1 17β-hydroxysteroid dehydrogenase: blocking of ER^+ breast cancer cell proliferation induced by estrone. *Bioorg. Med. Chem.* **2008**, *16*, 1849–1860.
26. Mazumdar, M.; Fournier, D.; Zhu, D.W.; Cadot, C.; Poirier, D.; Lin, S.X. Binary and ternary crystal structure analyses of a novel inhibitor with 17β-HSD type 1: a lead compound for breast cancer therapy. *Biochem. J.* **2009**, *424*, 357–366.
27. Maltais, R.; Tremblay, M.R.; Ciobanu, L.C.; Poirier, D. Steroids and combinatorial chemistry. *J. Comb. Chem.* **2004**, *6*, 443–456.
28. Ciobanu, L.C.; Maltais, R.; Poirier, D. The sulfamate functional group as a new anchor for solid-phase organic synthesis. *Org. Lett.* **2000**, *2*, 445–448.
29. Poirier, D.; Bérubé, M.; Fournier, D. In *Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Libraries (Peptides, Proteins and Nucleic Acids Small Molecule Organic Chemical Diversity*; Epton, R., Ed.; Mayflower Worldwide Limited: Kingswinford, UK, 2004.

30. Poirier, D.; Ciobanu, L.C.; Berube, M. A multidetachable sulfamate linker successfully used in a solid-Phase strategy to generate libraries of sulfamate and phenol derivatives. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2833–2838.
31. Ciobanu, L.C.; Poirier, D. Solid-phase parallel synthesis of 17 alpha-substituted estradiol sulfamate and phenol libraries using the multidetachable sulfamate linker. *J. Comb. Chem.* **2003**, *5*, 429–440.
32. Ciobanu, L.C.; Poirier, D. Synthesis of libraries of 16β-aminopropyl estradiol derivatives for targeting two key steroidogenic enzymes. *Chem. Med. Chem*. **2006**, *1*, 1249–1259.
33. Tremblay, M.R.; Poirier, D. Solid-phase synthesis of phenolic steroids: From optimization studies to a convenient procedure for combinatorial synthesuis of biologically relevant estradiol derivatives. *J. Comb. Chem.* **2000**, *2*, 48–65.
34. Peterson, E.M.; Brownell, J.; Vince, R. Synthesis and biological evaluation of 5'-sulfamoylated purinyl carbocyclic nucleosides. *J. Med. Chem.* **1992**, *35*, 3991–4000.
35. Carnazzi, E.; Aumelas, A.; Barberis, C.; Guillon, G.; Seyer, R. A new series of photoactivatable and iodinatable linear vasopressin antagonists. *J. Med. Chem.* **1994**, *37*, 1841–1849.
36. Bérubé, M.; Laplante, Y.; Poirier, D. Design, synthesis and *in vitro* evaluation of 4-androstene-3,17-dione/adenosine hybrid compounds as bisubstrate inhibitors of type 3 17beta-hydroxysteroid dehydrogenase. *Med. Chem.* **2006**, *2*, 329–347.
37. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y.Dicarbonates: convenient 4-dimethylaminopyridine catalyzed esterification reagents. *Synthesis* **1994**, 1063–1066.
38. Littke, A.F.; Schwarz, L.; Fu, G.C. A mild and general catalyst for Stille reactions of aryl chlorides and aryl bromides. *J. Am. Chem. Soc.* **2002**, *124*, 6343–6348.
39. Poirier, D.; Ciobanu, L.C.; Maltais, R. Steroid sulfatase inhibitors. *Exp. Opin. Ther. Patents* **1999**, *9*, 1083–1099.
40. Howarth, N.M.; Purohit, A.; Reed, M.J.; Potter, B.V.L. Oestrone 3-*O*-(*N*-acetyl)sulphamate, a potential molecular probe of the active site of oestrone sulphatase. *J. Med. Chem.* **1994**, *37*, 219–221.
41. Tremblay, M.R.; Lin, S.X.; Poirier, D. Chemical synthesis of 16β-propylaminoacyl derivatives of estradiol and their inhibitory potency on type 1 17β-hydroxysteroid dehydrogenase and binding affinity on steroid receptors. *Steroids* **2001**, *66*, 821–831.
42. Tremblay, M.R.; Poirier, D. Overview of a rational approach to design type I 17*β*-hydroxysteroid dehydrogenase inhibitors without estrogenic activity: chemical synthesis and biological evaluation. *J. Steroid Biochem. Mol. Biol*. **1998**, *66*, 179–191.
43. Dionne, P.; Nagatcha, B.T.; Poirier, D. D-ring allyl derivatives of 17β- and 17α-estradiols: Chemical synthesis and ^{13}C-NMR data. *Steroids* **1997**, *62*, 674–681.