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Synthesis and *in vitro* evaluation of 2-[^{11}C] methoxyestradiol-3,17β-*O*,*O*-bissulfamate for *in vivo* studies of angiogenesis

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In the present study, 2-methoxyestradiol-3,17β-*O*,*O*-bissulfamate (1), a known angiogenesis inhibitor, was prepared in a radiolabeled form by ^{11}C-methylation of 2-hydroxyestradiol-3,17β-*O*,*O*-bis(*N*-trityl)sulfamate (6) followed by detritylation. Synthesis of precursor 6 required a rather long step because of the presence of two sulfamoyl groups. The decay-corrected radiochemical yield of [^{11}C]1 was 19 ± 2% based on [^{11}C]CH_3I, and the specific activity was 34–39 GBq/μmol. Although 1 is known to significantly inhibit the proliferation of human umbilical vascular endothelial cells (HUVECs), its radiolabeled form, [^{11}C]1 was not avidly taken up by HUVECs, and the uptake increased slightly in a time-dependent manner (156% at 60 min relative to a value of 100% at 5 min). These results suggest that further studies are warranted to determine the molecular target for [^{11}C]1.

Keywords: 2-[^{11}C]methoxyestradiol-3,17β-bissulfamate; angiogenesis; carbonic anhydrase; steroid sulfatase

Introduction

2-Methoxyestradiol (2-ME), an endogenous estrogen metabolite, is known to inhibit angiogenesis. It has potent inhibitory activity against endothelial cell proliferation and migration *in vitro* and also inhibits the growth and neovascularization of solid tumors in mice by oral administration.[1] We previously synthesized 2-[^{11}C]ME (Figure 1) for *in vivo* angiogenesis studies, which showed high and specific uptake by human umbilical vascular endothelial cells (HUVECs) *in vitro* but low tumor uptake in C57BL/6 mice implanted with Lewis lung carcinoma (LLC) cells.[2] This result may be attributed to the rapid disappearance of 2-ME from the plasma after intravenous or oral administration.[3] Sulfamoylation of 2-ME, however, showed enhanced anti-proliferative effects compared with 2-ME on human breast cancer cells.[4] Among sulfamate derivatives, 2-ME-3,17β-*O*, *O*-bissulfamate (**1**) showed more potent anti-proliferative effects than 2-ME on MCF-7 and HUVECs by 10- and 60-fold, respectively.[4] On the basis of the *in vitro* data, **1** was further evaluated in mice implanted with LLC cells (5 mg/kg intraperitoneally, 10 mg/kg intraperitoneally, or 30 mg/kg intravenously) and in nude mice implanted with MDA-MB-435 cells (20 mg/kg, oral), and results showed significant inhibition of tumor growth in both models.[3,5] Moreover, **1** had favorable bioavailability (85%) and underwent little metabolism after intravenous or oral administration.[3]

Steroid sulfamates were initially found to be inhibitors of steroid sulfatase (STS).[6] Many tumors in which steroid sulfatase plays an important role, such as breast tumors, are hormone dependent because this enzyme converts estrogen sulfates to estrogens. Thus, steroid sulfamates may be good candidates for treatment of hormone-dependent tumors. Later, sulfamates were found to have inhibitory activity against carbonic anhydrases (CA), which catalyze the interconversion between CO_2 and bicarbonate, by binding of the sulfamate moiety to the catalytic zinc ion of the enzymes.[7] Some sulfamates are known to be dual inhibitors of CA and STS.[7,8] It was reported that 16α-fluoroestradiol-3,17β-bissulfamate is a potent inhibitor of human CAI (hCAI; IC_{50} = 288 nM) and hCAII (IC_{50} = 54 nM), which shows similar inhibitory activities to acetazolamide, a clinically used CA inhibitor (IC_{50} = 219 nM for hCAI and 30 nM for hCAII).[9] Biodistribution studies of 16α-[^{18}F]fluoroestradiol-3, 17β-bissulfamate demonstrated that this radioligand bound to both STS and CA in rats, tumor-bearing nude mice, and piglets; however, the binding affinities were not high enough for tumor imaging.[10,11]

It was reported that steroid sulfamate **1** has potent inhibitory effect on the proliferation of MCF-7 (IC_{50} = 250 nM) and HUVECs (IC_{50} = 50 nM) and also has inhibitory activity against hCAII (IC_{50} = 379 nM) as well as STS (IC_{50} = 39 nM).[4,8] In the present study, we synthesized 2-[^{11}C]methoxyestradiol-3, 17β-*O*,*O*-bissulfamate ([^{11}C]**1**) (Figure 1) and evaluated it *in vitro* for *in vivo* studies of angiogenesis.

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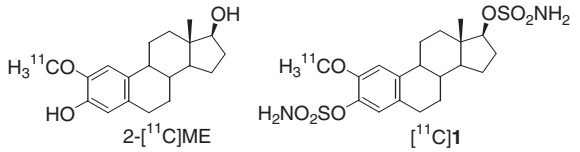


Figure 1. Structures of 2-[^{11}C]ME and 2-[^{11}C]ME-3,17 β-*O*,*O*-bissulfamate ([^{11}C]**1**).

Results and discussion

Chemistry

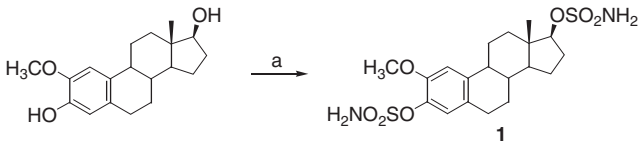
Compound **1** was synthesized using a previously described method (Scheme 1).[12,13] Synthesis of precursor **6** required a rather long step from 3,17β-estradiol because of the presence of two sulfamoyl groups at the C3 and 17β positions (Scheme 2). Introduction of a hydroxyl group to C2 of 3,17β-estradiol-3, 17β-*O*,*O*-bis(methoxymethyl)ether to give the 2-hydroxy compound **2** was carried out by boronylation using *s*-butyl lithium and trimethyl borate, followed by oxidation using sodium perborate.[14] It was reported that tritylated sulfamoyl groups are labile for sulfamoyl NH and trityl C bond cleavage under acidic conditions and for sulfamoyl O and sulfur bond cleavage with nucleophilic treatment.[15,16] Therefore, the hydroxyl group at the C2 position was first protected as benzyl ether, and then the methoxymethyl groups at the C3 and 17β positions were removed under acidic conditions. Sulfamoyl groups were introduced into the C3 and 17β positions using a procedure described in the literature,[12,17] and then the sulfamoyl amine groups were protected by tritylation prior to debenzylation at C2 position.

Radiochemical synthesis

Radiochemical synthesis of [^{11}C]**1** was carried out in a one-pot reaction (Scheme 2). Although ^{11}C labeling and purification were conducted using an automated system, the final reformulation step was carried out manually after the product eluted from the tC18 Sep-Pak cartridge using EtOH. However, we expect to conduct the whole process using an automated system if a higher dose of [^{11}C]CH_4 were to be used.

For the ^{11}C-methylation, use of base including NaH, DIPEA, or K_2CO_3 resulted in cleavage of the sulfamoyl groups of **6**. Therefore, NaOH, which was unreactive with the sulfamoyl groups, was the base of choice. Furthermore, reaction solvents (DMF, 2-butanone, or acetone) other than DMSO resulted in by-product formation. Removal of the trityl protecting groups from the sulfamoyl groups was carried out in 80% trifluoroacetic acid (300 μL) and 33% HBr-acetic acid (20 μL) (80 °C, 5 min). Although trityl groups are generally removed with trifluoroacetic acid, more harsh conditions were required for this one-pot reaction because the reaction mixture contained DMSO and NaOH.

The total synthesis time after [^{11}C]CH_4 production was 45–48 min. The decay-corrected radiochemical yield of [^{11}C]**1** was 19 ± 2% based on [^{11}C]CH_3I, and its specific activity was 34–39 GBq/μmol. Long-term use of [^{11}C]CH_4 target would increase the specific activity of the radioligand. Co-elution with **1** on HPLC confirmed the identity of [^{11}C]**1**.



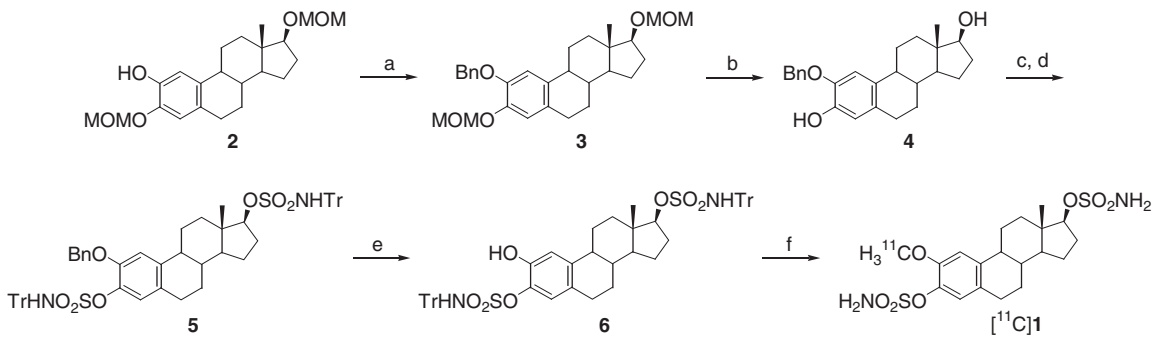
Scheme 1. Reagents and conditions: (a) H_2NSO_2Cl, DMA, room temperature, 1.5 h, 90%.

Partition coefficient

[^{11}C]**1** was found to have lower lipophilicity (log $P_{o/w}$ = 2.25) than 2-[^{11}C]ME (log $P_{o/w}$ = 2.95).[18]

Uptake of [^{11}C]1 by HUVECs

HUVECs are an *in vitro* model of angiogenesis, and **1** is known to inhibit HUVEC proliferation significantly.[4,19] Therefore, we investigated the uptake of [^{11}C]**1** by HUVECs. Small fraction of [^{11}C]**1** was found to be taken up by HUVECs (0.31% ID at 5 min and 0.48% ID at 60 min), and this uptake slightly increased in a time-dependent manner: 128 ± 4.6% at 15 min, 151 ± 5.3% at 30 min, and 156 ± 7.4% at 60 min (relative to a value of 100 ± 5.9% at 5 min) (Figure 2A). In addition, [^{11}C]**1** uptake by HUVECs at 60 min was inhibited in the presence of **1** (10 μM) by 23% (Figure 2B). In contrast, 2-[^{11}C]ME was taken up avidly by HUVECs (2.96% ID at 5 min and 6.9% ID at 60 min) and in a time-dependent manner (i.e., 160% at 15 min, 220% at 30 min, and 240% at 60 min; relative to a value of 100% at 5 min), and the uptake was inhibited in the presence of 2-ME by 70% at 60 min. However, tumor uptake of 2-[^{11}C]ME was not significantly high (1.04% ID/g) in C57BL/6 mice implanted with LLC cells.[2] This result



Scheme 2. Reagents and conditions: (a) K_2CO_3, acetone, benzyl bromide, 40 °C, 12 h, 93%; (b) 6 N HCl, THF, room temperature (rt), 7 h, 88%; (c) H_2NSO_2Cl, DMA, rt, 2 h, 75%; (d) trityl chloride, DIPEA, CH_2Cl_2, rt, 20 h, 56%; (e) H_2, 10% Pd/C, 1:5 THF–EtOH, rt, 6 h, 85%; (f) [^{11}C]CH_3I, 5 N NaOH, DMSO, 80 °C, 5 min; 80% trifluoroacetic acid, 33% HBr-acetic acid, 80 °C, 5 min.

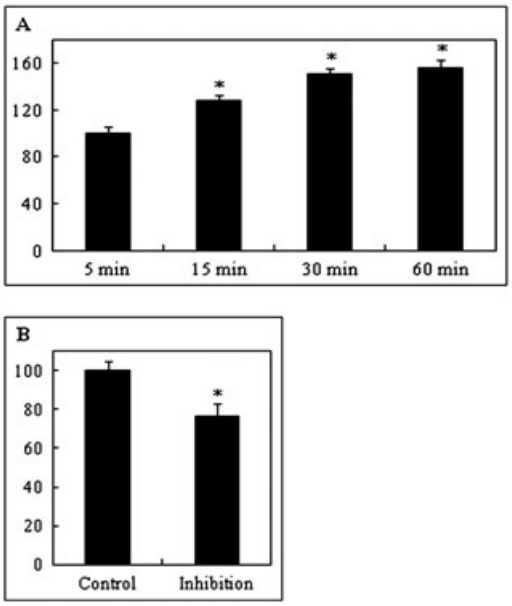


Figure 2. (A) Uptake of [^{11}C]**1** by HUVECs as a function of time. Uptake levels are expressed relative to a value of 100% at 5 min. (B) Inhibition of [^{11}C]**1** uptake by compound **1** in HUVECs after incubation for 60 min. Data are expressed as the mean of three measurements ± SD. *$P < 0.05$, compared to control level.

may be explained by the fact that 2-ME was rapidly removed from rat plasma 15 min after an intravenous injection.[3] It should be noted that **1** lasted much longer in rat plasma.[3] Ligand **1** has been known to have multiple targets for its antitumor activity, including inhibition of CA, STS, and angiogenesis. Therefore, it would be worthwhile to further investigate the molecular targets for [^{11}C]**1**, such as CA and STS.

Experimental

Materials and equipment

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). ^{1}H NMR spectra were obtained on a Varian ^{Unity}*Inova* 500NB (500 MHz) spectrometer (Palo Alto, CA, USA) at the Cooperative Center for Research Facilities, Sungkyunkwan University (Suwon, Korea). Chemical shifts (δ) are reported as ppm downfield from tetramethylsilane internal standard. Electron impact (EI) mass spectra were obtained on a JMS-700 Mstation (JEOL Ltd, Tokyo, Japan), and electrospray ionization (ESI) mass spectra were obtained on a LCQ-DECA-XP (Thermo Scientific, Waltham, MA, USA) at the Korea Basic Science Institute (Seoul, Korea). For radioligand purification and analysis, HPLC was conducted using a SpectraSystem (Thermo Scientific) equipped with a semi-preparative column (YMC-Pack C18, 5 μm, 10 × 250 mm) or an analytical column (YMC-Pack C18, 5 μm, 4.6 × 250 mm). The eluant was monitored simultaneously by UV (280 nm) and NaI(T1) radioactivity detectors.

[^{11}C]CH_4 was produced by the ^{14}N(p,α)^{11}C reaction using a cyclotron (GE Healthcare, Uppsala, Sweden), and the radiochemical synthesis of [^{11}C]**1** was carried out using a TRACERlab FXc Pro module (GE Healthcare, Uppsala, Sweden). Radioactivity was measured in a dose calibrator (Biodex Medical Systems, Shirley, NY, USA), and cell uptake was measured using a 2480 WIZARD^2 Automatic Gamma Counter (PerkinElmer, Waltham, MA, USA).

Chemistry

*Synthesis of 2-methoxyestradiol 3,17β-O,O-bissulfamate (**1**)*

2-Methoxyestradiol (25 mg, 0.083 mmol) in ice-cold DMA (400 μL) was slowly added to a solution of sulfamoyl chloride (1.05 mmol)[12] in DMA (400 μL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 1.5 h. The resulting mixture was diluted with water (1 mL) and extracted with ethyl acetate (1 mL × 2). The organic layer was washed with water and saturated NaCl and was dried over anhydrous Na_2SO_4. Flash column chromatography (1:1 hexane–ethyl acetate) yielded **1** as a white solid (34 mg) in 90% yield: ^{1}H NMR (DMSO-d_6) δ 7.80 (s, 2H), 7.38 (s, 2H), 6.99 (s, 2H), 4.35 (t, 1H, J = 8.5 Hz), 3.77 (s, 3H), 2.75–2.73 (m, 2H), 2.39–1.16 (m, 13H), 0.78 (s, 3H); MS (ESI) m/z 459.6 ($(M-H)^-$). The NMR and MS data agreed with literature values.[20]

*Synthesis of 2-hydroxyestradiol-3,17β-O,O-bis(methoxymethyl) ether (**2**)*

Compound **2** was synthesized according to a method described in the literature.[14] *s*-Butyl lithium (1.4 M in cyclohexane, 2.2 mL, 3.08 mmol) was slowly added to a solution of estradiol-3,17β-*O*,*O*-bis(methoxymethyl)ether (640 mg, 7.08 mmol) in THF (4 mL) at −75 °C under N_2, and the mixture was stirred at the same temperature for 2 h. Trimethylborate (804.5 μL, 7.08 mmol) was then slowly added, and the mixture was stirred at −75 °C for 15 min and then warmed to 0 °C. The reaction was quenched with 10% ammonium chloride (aq., 8 mL), and the mixture was stirred at room temperature for 1 h. Sodium perborate tetrahydrate (1.07 g, 7 mmol) was then carefully added in portions, and the mixture was stirred at room temperature for 15 h before filtering to remove inorganic salts. The filter cake was washed with ethyl acetate, and the filtrate was extracted with ethyl acetate. The combined organic layers were washed with saturated brine and were dried over anhydrous Na_2SO_4. Flash column chromatography (6:1 hexane–ethyl acetate) yielded **2** as a yellow oil (421 mg) in 63% yield: ^{1}H NMR ($CDCl_3$) δ 6.91 (s, 1H), 6.80 (s, 1H), 5.75 (s, 1H), 5.17 (s, 2H), 4.67 (ABq, 2H, J = 6.5 Hz), 3.63 (t, 1H, J = 8.5 Hz), 3.53 (s, 3H), 3.39 (s, 3H), 2.80–2.77 (m, 2H), 2.26–1.77 (m, 13H), 0.82 (s, 3H); MS (EI) m/z 376 (M^+); HRMS calculated for $C_{22}H_{32}O_5$ 376.2250, found 376.2250. The NMR and MS data agreed with literature values.[2,21]

*Synthesis of 2-O-benzyloxyestradiol-3,17β-O,O-bis(methoxymethyl) ether (**3**)*

Potassium carbonate (588.77 mg, 4.26 mmol) was added to compound **2** (321 mg, 0.852 mmol) in acetone (6 mL). The reaction mixture was stirred at room temperature for 10 min, and benzyl bromide (303.67 mL, 2.56 mmol) was slowly added. The resulting solution was stirred at 40 °C for 12 h, diluted with water (5 mL), and extracted with ethyl acetate (5 mL × 3). The combined organic layers were washed with water and saturated NaCl and were dried over anhydrous Na_2SO_4. Flash column chromatography (6:1 hexane–ethyl acetate) yielded **3** as a colorless oil (370 mg) in 93% yield. ^{1}H NMR (DMSO-d_6) δ 7.30–7.45 (m, 5H), 6.94 (s, 1H), 6.76 (s, 1H), 5.75 (s, 1H), 5.10 (ABq, 2H, J = 6.5 Hz), 5.06 (s, 2H), 4.58 (ABq, 2H, J = 6.5 Hz), 3.54 (t, 1H, J = 8.3 Hz), 3.37 (s, 3H), 3.25 (s, 3H), 2.71–2.68 (m, 2H), 2.26–1.16 (m, 13H), 0.74 (s, 3H); MS (EI) m/z 466 (M^+); HRMS calculated for $C_{22}H_{32}O_5$ 466.2719, found 466.2706.

*Synthesis of 2-O-benzyloxyestradiol (**4**)*

Compound **3** (266 mg, 0.57 mmol) was dissolved in THF (5.6 mL), and 6 N HCl (3.7 mL, 22.23 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 7 h, diluted with saturated sodium bicarbonate (5 mL), and extracted with ethyl acetate (4 mL × 2). The combined organic layers were washed with water (5 mL × 2) and then saturated NaCl and were dried over anhydrous Na_2SO_4. Flash column chromatography (2:1 hexane–ethyl acetate) yielded **4** as a colorless oil (189 mg) in 88% yield. ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.47–7.29 (m, 5H), 6.83 (s, 1H), 6.48 (s, 1H), 5.04 (s, 2H), 4.46 (s, 1H), 3.53–3.49(m, 1H), 2.65–2.59 (m, 2H), 2.21–0.85 (m, 13H), 0.66 (s, 3H); MS (EI) *m/z* 378 (M^+); HRMS calculated for $C_{25}H_{30}O_3$ 378.2195, found 378.2190.

*Synthesis of 2-O-benzyloxyestradiol-3,17β-O,O-bis(N-trityl)sulfamate (**5**)*

Compound **4** (184 mg, 0.486 mmol) in ice-cold DMA (5 mL) was slowly added to a solution of sulfamoyl chloride (10.5 mmol)[11] in DMA (5 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 2 h and then cooled to 0 °C. The resulting mixture was diluted with water (10 mL) and extracted with ethyl acetate (10 mL × 2). The organic layer was washed with water and saturated NaCl and was dried over anhydrous Na_2SO_4. Flash column chromatography (5:4 hexane–ethyl acetate) yielded 2-*O*-benzylestradiol-3,17β-*O*,*O*-bissulfamate as a white solid (195 mg) in 75% yield. ^{1}H NMR (DMSO-d_6) δ 7.86 (s, 2H), 7.50–7.31 (m, 7H), 7.03 (s, 2H), 5.15 (s, 2H), 4.34 (t, 1H, J = 8 Hz), 2.75–2.73 (m, 2H), 2.28–1.16 (m, 13H), 0.76 (s, 3H); MS (ESI) *m/z* 535.3 ($(M-H)^-$).

Bis-sulfamate (237 mg, 0.442 mmol) was dissolved in anhydrous dichloromethane (12 mL); DIPEA (923 mL, 5.29 mmol) was added; and the mixture was stirred at room temperature for 10 min. To the reaction mixture was added trityl chloride (369 mg, 1.32 mmol), and stirring was continued at room temperature for 20 h. After the reaction mixture was treated with saturated ammonium chloride (15 mL), the organic layer was washed with water and saturated NaCl and was dried over anhydrous Na_2SO_4. Flash column chromatography (3.5:1 hexane–ethyl acetate) yielded **5** as a white solid (518 mg) in 56% yield. ^{1}H NMR (DMSO-d_6) δ 9.09 (s, 1H), 8.71 (s, 1H), 7.43–7.22 (m, 35H), 6.96 (s, 1H), 6.64 (s, 1H), 5.05 (s, 2H), 4.19 (t, 1H, J = 8 Hz), 2.64–2.57 (m, 2H), 2.23–0.85 (m, 13H), 0.54 (s, 3H); MS (ESI) *m/z* 1019.3 ($(M-H)^-$).

*Synthesis of 2-hydroxyestradiol-3,17β-O,O-bis(N-trityl)sulfamate (**6**)*

A mixture of compound **5** (50 mg, 0.049 mmol) and 10% Pd/C (10 mg) in 1:5 THF–ethanol (2.4 mL) was stirred under a H_2 atmosphere at room temperature for 6 h. The reaction mixture was filtered, and the filtrate was diluted with water (3 mL) and extracted with ethyl acetate (3 mL × 2). The combined organic layers were washed with water and saturated NaCl and were dried over anhydrous Na_2SO_4. Flash column chromatography (3:1 hexane–ethyl acetate) yielded **6** as a white solid (39 mg) in 85% yield. ^{1}H NMR (DMSO-d_6) δ 9.12 (br s, 1H), 8.80 (br s, 1H), 8.75 (s, 1H), 7.35–7.22 (m, 30H), 6.78 (s, 1H), 6.55 (s, 1H), 4.18 (t, 1H, J = 8.5), 2.58–2.57 (m, 2H), 2.18–0.84 (m, 13H), 0.54 (s, 3H); MS (ESI) *m/z* 929.3 ($(M-H)^-$).

Radiochemical synthesis

$[^{11}C]CH_4$ was converted into $[^{11}C]CH_3I$ using I_2 at 740 °C. The resulting $[^{11}C]CH_3I$ was trapped in a mixture of **6** (4 mg, 4.30 μmol) and 5 N NaOH (7 μL, 35 μmol) in 300 μL of DMSO at 25 °C. This mixture was then stirred at 80 °C for 5 min and then treated with 80% trifluoroacetic acid (aq., 300 μL) and 33% HBr-acetic acid (20 μL) at 80 °C for 5 min. The solution was cooled to 40 °C, neutralized with 4 M sodium acetate (670 μL), and then injected onto a semi-preparative HPLC column, which was eluted with a 43:57 mixture of ethanol and water at a flow rate of 3.5 mL/min. The desired fraction, eluting at 12.8–13.8 min, was collected in a round flask and diluted with 30 mL of water. The product was trapped onto a tC18 Sep-Pak cartridge, which was rinsed with 10 mL of water and then with 1 mL of EtOH. The EtOH fraction was then transferred via a 1/16-in. tubing into a vial, which was placed in a drawer-type shielded port of the hot cell. The vial was then transferred to a separate hot cell. After EtOH was removed using a rotary evaporator, the product was redissolved in ethanol and was diluted with saline to give a final solution of 10% ethanol in saline. For specific activity determination, an aliquot of $[^{11}C]$**1** was injected onto an analytical column, which was eluted with a 55:45 mixture of ethanol and water at a flow rate of 0.8 mL/min (t_R = 8.2–9.0 min). Specific activity determinations were carried out by comparing the HPLC UV peak area of the desired radioactive peak with those of different concentrations of unlabeled compound **1**. An aliquot of $[^{11}C]$**1** was co-injected with **1** onto the HPLC system to confirm its identity.

Partition coefficient

Radioligand $[^{11}C]$**1** was added to premixed suspensions containing 600 μL of octanol and 600 μL of water, and the mixture was vortexed vigorously for 3 min and centrifuged at 2000 rpm for 5 min. After two layers had separated, 100-μL aliquots of the octanol and aqueous layers were removed and counted. Samples from the octanol and aqueous layers were repartitioned until consistent values were obtained. The experiment was carried out in triplicate. Log $P_{o/w}$ was expressed as the logarithm of the ratio of the counts per minute from octanol versus water.[22]

Uptake of $[^{11}C]$1 by HUVECs

HUVECs were cultured at 37 °C under 5% CO_2 in EBM-2 medium containing endothelial growth supplements, 10% fetal bovine serum, and antibiotics. The cells were grown to 80–90% confluence, harvested with trypsin, and washed twice with 1 mL of phosphate buffered saline (PBS, pH 7.4) containing 1% bovine serum albumin (BSA). Cells (10^5–10^6) were subsequently transferred into Eppendorf tubes. Radioligand $[^{11}C]$**1** (555 kBq/5 μL) was dissolved in 10% ethanol–saline and incubated with HUVECs (50 μL of PBS, pH 7.4) at 37 °C for 5, 15, 30, and 60 min (n = 3). Cells were then washed twice with PBS (pH 7.4) containing BSA (1%), and counted.

To investigate inhibition of $[^{11}C]$**1** uptake by **1**, HUVECs were incubated with $[^{11}C]$**1** (555 kBq/5 μL) in the presence of 10 μM of **1** (5 μL in 10% ethanol–saline) at 37 °C for 60 min. Cells were then washed as described above and counted.

All experiments were performed in triplicate, and the results were analyzed using unpaired two-tailed Student's *t*-test. Differences at the 95% confidence level ($P < 0.05$) were considered significant.

Conclusions

Steroid sulfamate $[^{11}C]$**1** was prepared by ^{11}C-methylation of the 2-hydroxy precursor followed by detritylation. Synthesis of the 2-hydroxy precursor required seven steps from 3,17β-estradiol. Although **1** is known to significantly inhibit the proliferation of

HUVECs, the low uptake of [^{11}C]**1** by HUVECs suggests that further studies are warranted to determine the molecular target for [^{11}C]**1**.

Acknowledgements

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