

Inhibition of Estrone Sulfate-induced Uterine Growth by Potent Nonestrogenic Steroidal Inhibitors of Steroid Sulfatase¹

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ABSTRACT

The present study describes the biological *in vitro* and *in vivo* evaluation of 2-methoxy derivatives of estrogen inhibitors of steroid sulfatase, namely 3-sulfamoyloxy-17 α -*p*-*tert*-butylbenzyl(or benzyl)-1,3,5 (10)-estratrien-17 β -ols. The addition of the 2-methoxy group conserves the potent inhibitory effect on steroid sulfatase activity (IC₅₀s of 0.024 and 0.040 nM) while removing the estrogenic action. Using an ovariectomized mouse model, we show that the first generation of steroid sulfatase inhibitors tested, 3-sulfamoyloxy-17 α -*p*-*tert*-butylbenzyl(or benzyl)estra-1,3,5 (10)-trien-17 β -ols and estrone-3-*O*-sulfamate, are estrogenic compounds stimulating estrogen-sensitive uterine growth. Interestingly, the 2-methoxy-3-sulfamoyloxy-17 α -benzylestra-1,3,5 (10)-trien-17 β -ol (7) has no estrogenic activity but efficiently blocks (*s.c.* and *p.o.*) uterine growth induced by estrone sulfate, which is converted into estrone and then estradiol by steroid sulfatase and type 1 17 β -hydroxysteroid dehydrogenase, respectively. This report clearly shows that a steroid sulfatase inhibitor can efficiently block estrogen action from the inactive precursor estrone sulfate, *in vitro* and *in vivo*.

INTRODUCTION

E₂³ is the main steroid hormone supporting growth of tumors in patients with estrogen-sensitive breast cancer (1). In addition to the blockade of E₂ action that can be achieved by the use of antiestrogens (2, 3), a complementary approach to reduce the effects of estrogens consists in the inhibition of steroidogenic enzymes involved in their synthesis (4). Thus, extensive work has focused over the years on the development of inhibitors of the steroidogenic enzymes, namely aromatase (5–7), 17 β -HSDs (8, 9), and steroid sulfatase (10–12). Inhibition of aromatase has been shown to provide an efficient blockade of the synthesis of potent estrogens, estrone (E₁), and E₂ (13–16) but cannot block the effect of weak estrogen 5-diol (17–19). The enzymes steroid sulfatase and type 1 17 β -HSD mediate the synthesis of 5-diol from the adrenal precursors DHEAS and dehydroepiandrosterone available in the circulation. In addition, transformation of sulfated steroid estrone sulfate (E₁S), the most abundant C18 steroid in aged women, by steroid sulfatase and type 1 17 β -HSD provides another major source of potent estrogens E₁ and E₂ in breast tissue (4, 20–22).

There is now evidence that an important amount of E₂ in breast tumors originates from local transformation of E₁S following the sulfatase pathway, whereas <10% results from the aromatization of androst-4-ene-3,17-dione and testosterone (23, 24). The same syn-

thetic pathway accounts for the transformation of DHEAS into the estrogenic C19 steroid 5-diol. Thus, inhibition of steroid sulfatase, the enzyme responsible for the conversion of DHEAS to dehydroepiandrosterone and E₁S to E₁, may allow reduction of estrogen levels in tumors and could represent a promising approach for the treatment of estrogen-sensitive breast cancer.

Some of the most efficient steroid sulfatase inhibitors developed were sulfamate derivatives (10–12). The first reported inhibitor in this series, estrone sulfamate (EMATE; Ref. 25, 26) is a very potent irreversible inhibitor, but it is also an estrogenic compound (27), thus having less than optimal characteristics for the therapy of estrogen-sensitive cancers. Purohit *et al.* (28) then investigated various A-ring substituted analogues of EMATE and found that the 2-methoxy derivative was nonestrogenic and yet a potent inhibitor. Studies reported previously by other groups have in fact indicated that the E₂ metabolite 2-methoxy-E₂ has reduced ER binding affinity (29) as well as interesting antiangiogenic, antiproliferative, apoptotic, anticarcinogenic, and cytotoxic properties (30–35). Our group has shown previously that the combined effects of a benzyl (or *tert*-butylbenzyl) group at C17 α and a sulfamate group at C3 of E₂ provide an improved inhibition of steroid sulfatase activity (36, 37). These inhibitors, however, induced the proliferation of estrogen-sensitive ZR-75-1 cells, thus suggesting an estrogenic activity (38). We then concluded that the 2-methoxy analogues of these inhibitors might be nonestrogenic and possibly promising therapeutic agents for estrogen-sensitive cancers by targeting important actions: (a) the inhibition of steroid sulfatase; (b) the inhibition of ER⁺ cell proliferation; and (c) the inhibition of tumor angiogenesis (Fig. 1). Herein, we present the chemical synthesis briefly, the steroid sulfatase *in vitro* and *in vivo* inhibitory activities, as well as an *in vivo* study on the estrogenic properties of these new steroidal inhibitors.

MATERIALS AND METHODS

Chemical Synthesis. Starting steroids 2-methoxyestrone (1) and 2-methoxy-3-*O*-benzylestrone (2) were purchased from Steraloids, Inc. (Newport, RI) or synthesized similarly as reported in literature (39). Target compounds 6 and 7, as well as intermediate compounds 3–5, were synthesized from 1 and 2 as exemplified in Fig. 2 with few modifications of the procedure used for the synthesis of 11–14 (37). Additional compounds 8–10 were synthesized using classical reduction or sulfamoylation reactions already reported in the preparation of similar compounds (37, 38, 40). The chemical structure of all compounds were fully characterized by infrared, NMR, and mass analysis, and the purity of tested compounds was determined by HPLC and found acceptable for biological assays with purities ranging from 97 to 99%. The next data reported for key compound 7 as well as the HPLC profile (Fig. 3) are representative of all compounds tested in our study. The full details of the experimental procedure and characterization will be available on request addressed to the corresponding author.

2-Methoxy-3-sulfamoyloxy-17 α -benzylestra-1,3,5 (10)-trien-17 β -ol (7). White solid; infrared (KBr): 3395 (OH and NH₂); ¹H NMR (CDCl₃): 0.98 (s, 18-CH₃) 1.3–2.4 (14H), 2.68 and 2.94 (2d of AB-system, *J* = 13.2 Hz, CH₂Ph), 2.82 (m, 6-CH₂), 3.89 (s, OCH₃), 4.96 (s, OSO₂NH₂), 6.96 (s, 4-CH), 7.06 (s, 1-CH), 7.33 (m, CH₂Ph); ¹³C NMR (CDCl₃): 14.44 (18-C), 23.27 (15-C), 26.36 and 27.28 (7- and 11-C), 28.62 (6-C), 31.36 (12-C), 33.7 (16-C), 39.11 (8-C), 42.33 (1'-C), 44.34 (9-C), 46.69 (13-C), 49.53 (14-C), 56.35 (OCH₃), 82.98 (17-C), 110.44 (1-C), 124.06 (4-C), 126.36 (5'-C), 128.14 (3'' and 5''-C), 130.19 (5-C), 131 (2'' and 6''-C), 136.8 (10-C), 138.12 (1'-C),

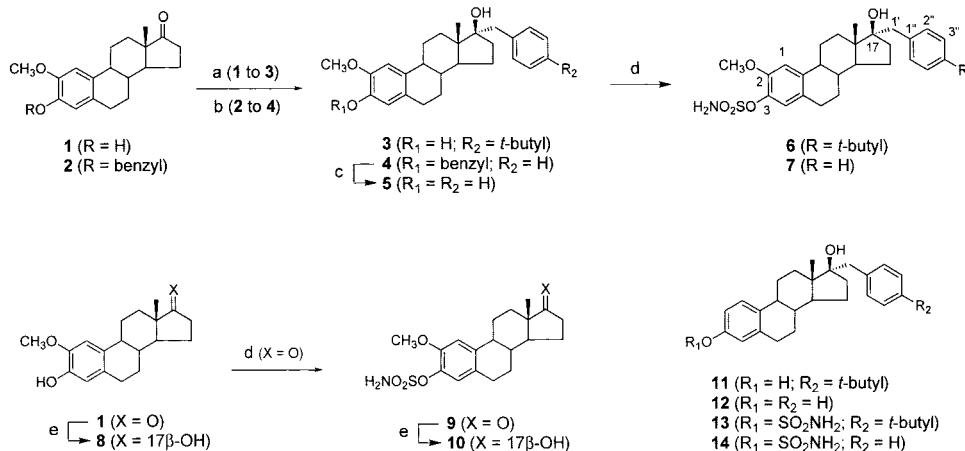
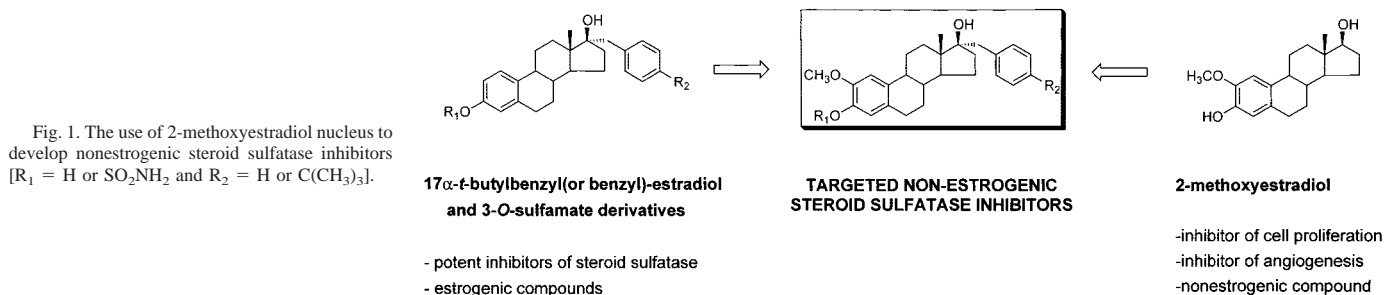
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³ The abbreviations used are: E₂, estradiol; HSD, hydroxysteroid dehydrogenase; NMR, nuclear magnetic resonance; 5-diol, androst-5-ene-3 β ,17 β -diol; ER, estrogen receptor; OVX, ovariectomized; HPLC, high-performance liquid chromatography; DHEAS, dehydroepiandrosterone sulfate; CMV, cytomegalovirus; HEK, human embryonic kidney.



140.41 (3-C), 148.93 (2-C); LRMS for C₂₆H₃₃NO₅S + NH₄ [MNH₄⁺]: 489.6 *m/z*; HPLC purity = 97.2% (C-18 Nova Pak column, H₂O: methanol/30:70).

Steroid Sulfatase Assays (*in Vitro* Studies). HEK-293 cells (American Type Culture Collection, Rockville, MD), transiently transfected with a sulfatase expression vector (pCMV-sulfa), were used as the source of steroid sulfatase activity. The pCMV-sulfa was constructed by insertion of a cDNA fragment, downstream of the CMV promoter of the pCMV vector, kindly provided by Dr. M. B. Mathews (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY). The sulfatase cDNA fragment was obtained by screening of a human placenta cDNA library (Clontech Laboratories Inc., Palo Alto, CA) using the incomplete cDNA fragment kindly provided by Dr. L. J. Shapiro (Howard Hughes Medical Institute, Los Angeles, CA) as probe. Transfection of the expression vector was performed by the calcium phosphate procedure using 10 μ g of recombinant plasmid/10⁶ cells. The cells were initially plated at 10⁴ cells/cm² in Falcon culture flasks and grown in DMEM containing 10% (volume for volume) fetal bovine serum supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 IU penicillin/ml, and 100 μ g of streptomycin sulfate/ml. For assay, the HEK-293 cells transfected with steroid sulfatase activity were prepared by repeated freezing (-80°C), thawing (five times), and homogenization using a Dounce homogenizer. The enzymatic reaction was carried out at 37°C for the transformation of [³H]E₁S into [³H]E₁ in 1.25 ml of 100 μ M Tris-acetate buffer (pH 7.4) containing 5 mM EDTA, 10% glycerol, 100 μ M of radioactive substrate ([6,7-³H]estrone sulfate ammonium salt; New England Nuclear, Boston, MA), and an ethanolic solution of compound to test (at appropriate concentrations). About 2.2 mg protein were used in the tests. After 2 h of incubation, the reaction was stopped by the addition of 1.25 ml of xylene. The tubes were then stirred and centrifuged at 2500 rpm for 10 min to separate organic and aqueous phases. Radioactivities in 750 μ l of each phase (organic: [³H]E₁ and aqueous: [³H]E₁S) were recorded by liquid scintillating counting with a Beckman LS3801 (Irvine, CA). The results are reported as a percentage (%) of inhibition at a fixed concentration of tested compounds (Table 1) or as an IC₅₀ (Fig. 4).

Steroid Sulfatase and Estrogenicity Assays (*in Vivo* Studies). Female BALB/c mice (BALB/cAnNCrlBR) weighing 18–20 grams were obtained from Charles River, Inc. (St-Constant, Québec, Canada) and housed four to five per cage in a temperature (22 \pm 3°C) and light (12 h/day, lights on at 7h15) controlled environment. The mice were fed rodent chow and tap water *ad libitum*. The animals were OVX under isoflurane-anesthesia via bilateral flank incisions and randomly assigned to groups of 8–10 animals. Ten mice were kept intact (INT) as

control. To exert an estrogenic effect and stimulate an estrogen-sensitive parameter as uterus, E₁S is converted into E₁ and then into E₂, under the catalytic activities of the enzymes steroid sulfatase and type 1 17 β -HSD, respectively. Mice in the intact and OVX control groups received the vehicle alone (8% ethanol-0.4% methylcellulose) during the 9-day period. The possible estrogenic activity (Fig. 5) of tested compounds was evaluated after their administration by subcutaneous (s.c.) injection [100 μ g, s.c., once daily (ID)] alone to OVX female mice for 9 days. For the evaluation of the inhibition of steroid sulfatase activity (Figs. 6–8), the tested compounds were administered as suspension in 8% ethanol-0.4% methylcellulose to OVX mice for 9 days (1, 10, or 100 μ g s.c. or oral (p.o.), ID, from day 2 to 10 of the study). The mice were simultaneously treated with E₁S [2 μ g, s.c., twice daily (BID)] from day 5 to 10 of the study. The tested compounds were also administered to OVX mice treated simultaneously with E₁ (0.06 μ g, s.c., BID) from day 5 to 10 of the study (Fig. 6). This was needed to ensure that the effect observed on the uterine weight does not result from an antiestrogenic effect instead of the inhibition of steroid sulfatase. On day 11, the mice were sacrificed by exsanguination followed by cervical dislocation. Uterus from mice were rapidly dissected, weighed, and kept in 10% buffered formalin for further histological

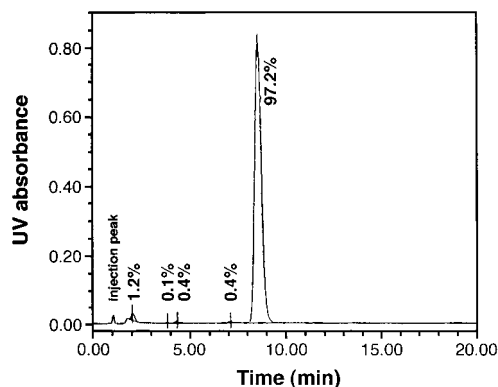


Fig. 3. HPLC profile of key compound **7**. The experiment was performed with a C18 Nova Pak reversed phase column (150 \times 3.9 mm), a mixture of H₂O:methanol/30:70 as eluent, and a UV detector (205 nm).

Table 1 *In vitro* inhibition of steroid sulfatase activity (homogenates of transfected HEK-293 cells) by 2-methoxy derivatives and reference inhibitors^a

| Compound ^b | C3-group | C2-group | C17 α -group | Inhibition (%) at 30 nM ^c | Inhibition (%) at 300 nM ^c | Inhibition (%) at 3000 nM ^c | IC ₅₀ (nM) |
|-----------------------|-----------|----------|------------------------|--------------------------------------|---------------------------------------|--|-----------------------|
| 3 | Phenol | Methoxy | <i>t</i> -Butyl-benzyl | 52 | 86 | 95 | 28 \pm 9 |
| 5 | Phenol | Methoxy | Benzyl | 0 | 15 | 63 | |
| 6 | Sulfamate | Methoxy | <i>t</i> -Butyl-benzyl | 99 | 99 | 99 | 0.040 \pm 0.002 |
| 7 | Sulfamate | Methoxy | Benzyl | 99 | 99 | 99 | 0.024 \pm 0.008 |
| 8 | Phenol | Methoxy | H | 0 | 0 | 0 | |
| 9 | Sulfamate | Methoxy | Ketone | 89 | 98 | 99 | 1.70 \pm 0.16 |
| 10 | Sulfamate | Methoxy | H | 80 | 97 | 99 | |
| 11 | Phenol | H | <i>t</i> -Butyl-benzyl | 91 | 97 | 97 | |
| 12 | Phenol | H | Benzyl | 19 | 66 | 92 | |
| 13 | Sulfamate | H | <i>t</i> -Butyl-benzyl | 99 | 99 | 99 | 0.030 \pm 0.003 |
| 14 | Sulfamate | H | Benzyl | 99 | 99 | 99 | |

^a Inhibition of the transformation of [³H]E₁S (100 μ M) to [³H]E₁.

^b See Fig. 2 for the chemical structures of tested compounds.

^c Error estimated at \pm 5%.

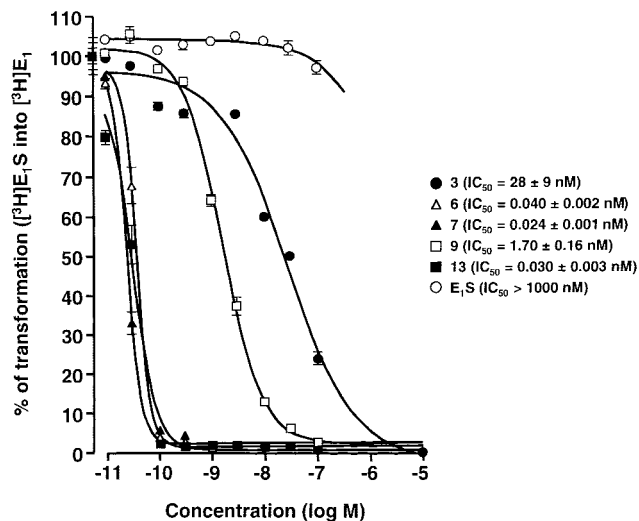


Fig. 4. Effects of increasing concentration of compounds 3, 6, 7, 9, 13, and E₁S on enzymatic activity of steroid sulfatase transfected in HEK-293 cells (see "Materials and Methods" for details). The results are expressed as a percentage of transformation of [³H]E₁S into [³H]E₁. Data points represent the mean values \pm SE from triplicate incubations.

examination. Data are expressed as the means \pm SE, and statistical significance was determined according to the multiple range test of Duncan-Kramer (41).

RESULTS AND DISCUSSION

***In Vitro* Study (Steroid Sulfatase Inhibitory Activity).** The inhibition of steroid sulfatase activity by 2-methoxylated compounds 3, 5–10 and reference compounds 11–14 was investigated in homogenate of transfected HEK-293 cells transforming E₁S to E₁ (Table 1) following a procedure reported previously (37). In the phenol series, the 2-methoxy-E₂ (8) does not inhibit steroid sulfatase activity. In the case of other 2-methoxy derivatives 3 and 5, the methoxy group at C2 is responsible for a reduced inhibitory action compared with that of the parent compounds 11 and 12. This is probably caused by the activating effect induced by the *ortho*-positioning of the methoxy group, resulting in lower acidity of the phenolic group at C3. Thus, at a concentration of 300 nM, compounds 3 and 5 with a *tert*-butylbenzyl or a benzyl group at C-17 α inhibited 86 and 15% of the enzyme activity, respectively, whereas their analogues 11 and 12 without a 2-methoxy group were more potent with 97 and 66% inhibitions. These differences were even more pronounced in the test run with the same compounds at a concentration of 30 nM. In the sulfamate series, all of the sulfamate derivatives inhibited >80% of the enzyme activity at 30 nM. At this concentration, the use of a *tert*-butylbenzyl (or benzyl) group at C17 α provided a stronger inhibition of the enzyme, and the potency of inhibitors 6 and 7 was found higher than

that of 2-methoxy-EMATE (9) or 2-methoxy-E₂-3-*O*-sulfamate (10). Thus, our first observation (37, 38) about the complementarity of the inhibitory effects of a sulfamate group at C3 and a suitable hydrophobic group at C17 was confirmed here again with the 2-methoxylated compounds. Moreover, compounds 6, 7, 13, and 14 with two inhibiting groups fully inhibited (99%) enzymatic activity and were more active compared with their analogues without the sulfamate group at C3, namely compounds 3, 5, 11, and 12.

The methoxy derivatives 3, 6, 7, and 9 and the reference inhibitor 13 (37) were next tested at various concentrations to determine the IC₅₀s for inhibition of steroid sulfatase activity. The results presented in Fig. 4 illustrate that all of these compounds are potent inhibitors of the enzyme. In our test, the IC₅₀ of 2-methoxy-EMATE (9) was 1.7 nM, whereas compounds 6, 7, and 13 with the two inhibiting groups *tert*-butylbenzyl (or benzyl) and sulfamate were much more potent (IC₅₀ = 0.04, 0.024, and 0.03 nM). The nonsulfamoylated inhibitor 3 was also a good inhibitor with an IC₅₀ in the range of 30 nM. Although the introduction of the methoxy group at C2 resulted in a decrease of the inhibitory activity, this decrease can however be considered as small. Indeed, the IC₅₀s of the reference inhibitor 13 and its 2-methoxy analogue 6 were 0.03 and 0.04 nM, respectively. In a previous test performed by Purohit *et al.* (28), the IC₅₀ of 2-methoxy-EMATE (9) in placental microsomes was found to be \geq 7-fold beyond that of EMATE. Rather interestingly, with an IC₅₀ of 0.024 nM, compound 7 was apparently the best inhibitor of steroid sulfatase within those tested in our assay. Compound 7 was also tested on type 1 17 β -hydroxysteroid dehydrogenase, and no inhibition of E₁ into E₂ transformation was observed at the two concentrations of 0.1 and

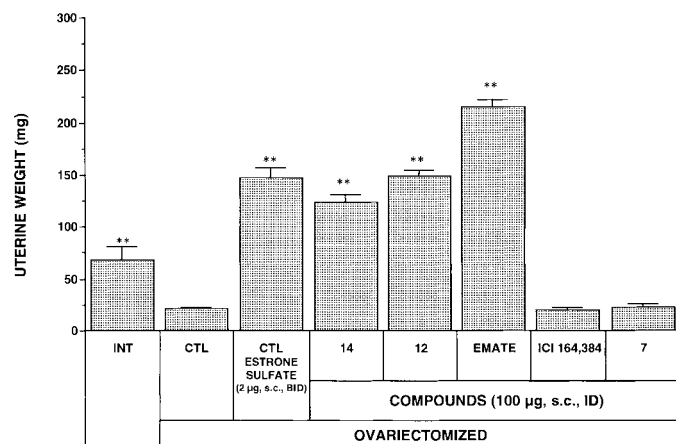


Fig. 5. Effect of estrone sulfate (E₁S), steroid sulfatase inhibitors (7, 12, 14, and EMATE), and a pure antiestrogen (ICI 164,384) on the uterine weight of OVX mice (***P* < 0.01, experimental *versus* OVX control animals). *INT*, intact mice. *CTL*, control group.

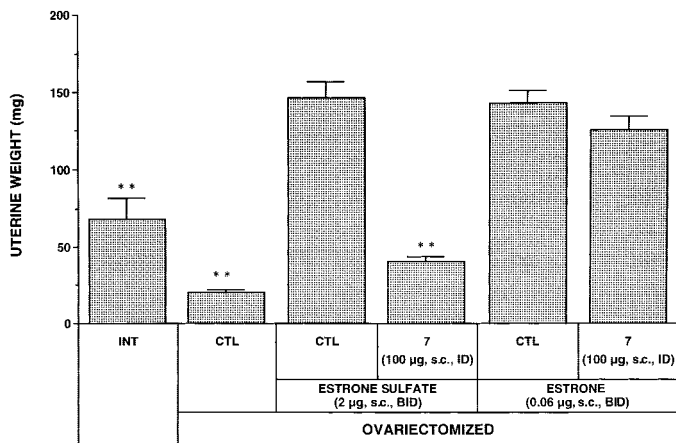


Fig. 6. Effect of steroid sulfatase inhibitor **7** on uterine weight of OVX mice from groups treated with estrone sulfate (E₁S) and estrone, respectively. (***P* < 0.01, experimental versus OVX + E₁S control animals). INT, intact mice; CTL, control group.

1 µM.⁴ After a preliminary evaluation of the biodisponibility of the tested compounds, the benzyl derivative **7** was selected for additional *in vivo* studies focusing on antisulfatase and estrogenic activities.

In Vivo Studies. Estrogenic, antiestrogenic, and steroid sulfatase inhibitory (antisulfatase) activities of tested compounds were investigated *in vivo* using the OVX mouse model. As shown in Figs. 5–8, ovariectomy induces a 70% decrease of uterine weight because of deprivation of estrogens. When administered to OVX mice, E₁S is converted in the uterine tissue into E₁ by the steroid sulfatase and then into E₂ by type 1 17β-hydroxysteroid dehydrogenase. The increase of uterine weight, an estrogen-sensitive tissue, then reflected the formation of active estrogens from E₁S.

To discriminate between the estrogenic or nonestrogenic activities of a series of steroid sulfatase inhibitors, compounds **7**, **12**, **14**, EMATE, and ICI 164,384 were injected s.c. to OVX female mice in the absence of treatment with E₁S (Fig. 5). No significant response or estrogenic stimuli of the uterus was observed with the pure antiestrogen ICI 164,384 as well as with our selected 2-methoxylated inhibitor, compound **7**, at the high dose of 100 µg. In contrast, the parent inhibitors **12**, **14**, and EMATE showed full estrogenic activity as reported previously *in vitro* (38, 27). Thus, none of these three later inhibitors can be used as therapeutic agents for the treatment of estrogen-sensitive breast cancer.

In the experiment reported in Fig. 6, E₁S or E₁ were injected s.c. to OVX mice simultaneously to the administration of the selected inhibitor **7** at the daily dose of 100 µg. The uterine weight increase induced by E₁S was strongly inhibited (84%) by the nonestrogenic steroid sulfatase inhibitor **7**, whereas the uterine weight induced by E₁ was not significantly decreased (14%, nonsignificant). These results obtained with E₁ and E₁S clearly demonstrate that compound **7** does not act as an antiestrogen (by blocking ER) but rather is a potent steroid sulfatase inhibitor efficiently blocking the transformation of E₁S into active estrogens.

As shown in Fig. 7, the inhibitors **12**, **14**, and EMATE (all without a methoxy group) reported previously did not reduce uterine weight when compared with control (OVX + E₁S), thus indicating their estrogenic properties. The administration of a nonestrogenic steroid sulfatase inhibitor should block estrogen synthesis from E₁S, and, correspondingly, the uterine weight increase induced by E₁S will be prevented. Nevertheless, an estrogenic inhibitor of the steroid sulfatase can itself exert an inhibitory action on steroid sulfatase (reducing

uterine weight), but, simultaneously, it stimulates the growth of the uterus. In the same experiment, the antiestrogen ICI 164,384 induces a dose-dependent decrease of the uterine weight. Santner and Santen have reported previously the inhibition of estrone sulfatase by the antiestrogen ICI 164,384 in rat mammary tumors (42). However, with a K_i of 11 µM, this was a weak inhibitor. The effect of ICI 164,384 in OVX mice simultaneously treated with E₁S (30 and 91% at 10 and 100 µg) is predominantly attributable to its antiestrogenic activity, rather than to antisulfatase activity. In contrast to other tested inhibitors, compound **7**, the 2-methoxy derivative of **14**, reduced by >84% the uterine growth induced by E₁S at both doses of 10 and 100 µg. A similar inhibitory effect (72%) was also observed following the administration of compound **7** at a lower dose of 1 µg, s.c. (Fig. 8). This later compound was also tested at a 100-µg dose administered p.o., and the inhibition achieved was the same as obtained with a 10-µg dose injected s.c. (83 and 80%, respectively). In summary, the *in vivo* experiments with the uterine weight model clearly prove that the synthesized compound **7** has no antiestrogenic activity and is a nonestrogenic potent inhibitor of steroid sulfatase activity, both following parenteral and p.o. administration.

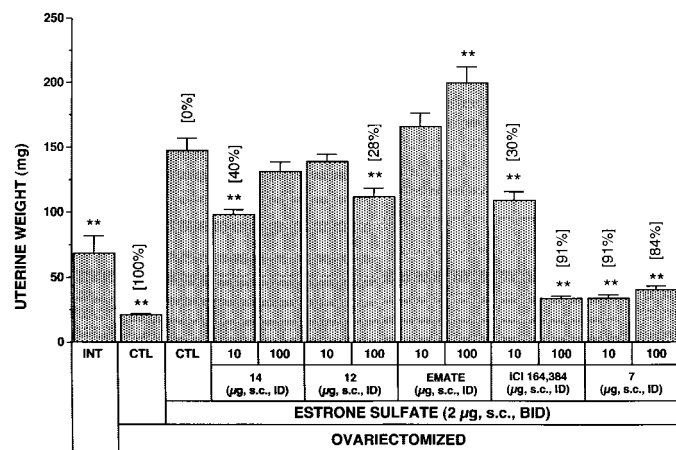


Fig. 7. Effect of steroid sulfatase inhibitors (**7**, **12**, **14**, and EMATE) and a pure antiestrogen (ICI 164,384) on the uterine weight of OVX mice after a simultaneous administration of estrone sulfate (E₁S). Results allow to compare the antisulfatase potency of the tested inhibitors at two different doses (***P* < 0.01, experimental versus OVX + E₁S control animals). INT, intact mice; CTL, control group. Data in brackets represent the reduction (%) of induced uterine weight (CTL + E₁S + OVX).

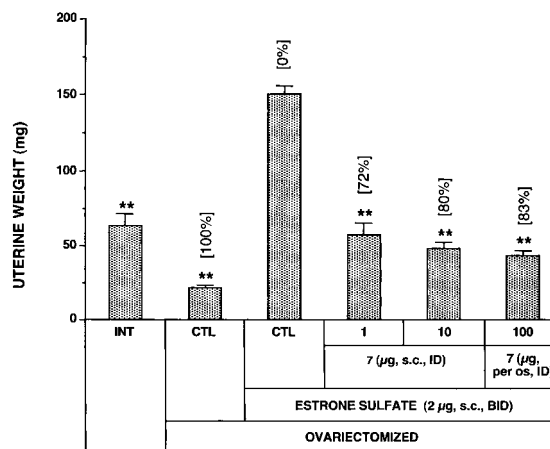


Fig. 8. Effect of steroid sulfatase inhibitor **7**, injected s.c. or administered p.o., on the uterine weight of OVX mice receiving simultaneously estrone sulfate (E₁S). ***P* < 0.01, experimental versus OVX + E₁S control animals. INT, intact mice; CTL, control group. Data in brackets represent the reduction (%) of induced uterine weight (CTL + E₁S + OVX).

⁴ V. Luu-The, unpublished data.

The results obtained from our *in vivo* study are in agreement with previous reports indicating that EMATE is an estrogenic compound that could not be used in the treatment of estrogen-sensitive cancers (27, 28). Our results also demonstrate that the reversible inhibitor 17 α -benzyl-E₂ (12) and the irreversible inhibitor 3-*O*-sulfamate-17 α -benzyl-E₂ (14) are both estrogenic in the OVX mouse model (uterine weight). At the opposite and more interestingly, the 3-*O*-sulfamate-2-methoxy-17 α -benzyl-E₂ (7), which is the 2-methoxylated analogue of 14, was found to be a potent steroid sulfatase inhibitor, nonestrogenic, and without anti-estrogenic activity (no effect mediated by ER). This compound reverses very efficiently the uterine weight stimulation normally induced by E₁S after its transformation into active estrogens by blocking the steroid sulfatase. Compound 7 is also a potent *in vitro* inhibitor of steroid sulfatase with an IC₅₀ of 0.024 nM for the transformation of E₁S to E₁ in homogenate of transfected HEK-293 cells. Because of the combined effects of the sulfamate and benzyl (or *tert*-butylbenzyl) groups introduced at C3 and C17 α (37), the potency of 6 and 7 are comparable with that of the parent compound without the 2-methoxy group. Because it was shown previously that estrogen metabolite 2-methoxy-E₂ (8) has low binding affinity for the estrogen receptor, exerts cytotoxic action in cancer cell cultures, and inhibits tumor angiogenesis (29–35, 43), we thus expect that the targeted 2-methoxylated inhibitors reported above might conserve some of these properties in addition to their very potent inhibition of steroid sulfatase. Additional studies will however be necessary to confirm this point as well as to focus on the use of these inhibitors in the therapy of estrogen-sensitive cancers.

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