Estrone Sulfamates: Potent Inhibitors of Estrone Sulfatase with Therapeutic Potential

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Breast cancer is a major cause of death for women in western countries, and estrogens have a central role in the development and growth of breast tumors.[1,2] In postmenopausal women, in whom breast cancer most frequently occurs, concentrations of estrone (1) (Figure 1) and estradiol are higher than in normal breast tissue and blood.[3] As concentrations of estrogens are similar in estrogen receptor positive and negative tumors, *in situ* formation of estrogen from estrogen precursors is likely to make a major contribution to the estrogen content of breast tumors.[4]

The enzymes required for the formation and interconversion of biologically potent estrogens (i.e., aromatase, estradiol dehydrogenase, and estrone sulfatase) are present in normal and malignant breast tissues.[5] Specific inhibitors of estrogen synthesis should therefore offer an important advance in the treatment available for breast cancer, and so far the greatest attention has been focused on the development of inhibitors of aromatase activity. However, although compounds like aminoglutethimide and 4-hydroxyandrostenedione greatly reduce peripheral aromatase activity, plasma estrone and estrone sulfate concentrations are only reduced by about 50%.[6,7] Plasma concentrations of estrone sulfate (2) (Figure 1) are much higher than those of unconjugated estrone,[8] and estrone sulfatase activity, which is responsible for the conversion of estrone sulfate to estrone, is a million times greater than aromatase activity,[5] which converts androstenedione to estrone, in breast tissues. In breast tumors 10 times more estrone originates via the sulfatase than aromatase pathway,[9] suggesting that the estrone sulfatase pathway may be the primary mechanism for the *in situ* production of estrogens in such tumors. Although the K_m for estrone sulfatase in breast tumors is about 20 μM[10] and substrate levels are only in the 1–10 nM range, concentrations as low as 1–10 pM can stimulate breast cancer cell growth.[11] Consequently, it is possible that inhibitors of estrone sulfatase, used alone or together with an aromatase inhibitor, may enhance the response to this form of endocrine therapy.

In contrast to aromatase inhibitors, the development of estrone sulfatase inhibitors is still at a very early stage. Previously, we have reported that the synthetic analogue estrone 3-*O*-methylthiophosphonate (3) (E1-3-MTP)[10,12] as well as a wider range of estrone 3-*O*-phosphonate and thiophosphonate analogues[12] inhibit the hydrolysis of estrone sulfate efficiently in MCF-7 breast cancer cells and in placental and breast tumor preparations. We now report the synthesis and inhibitory activity of a different

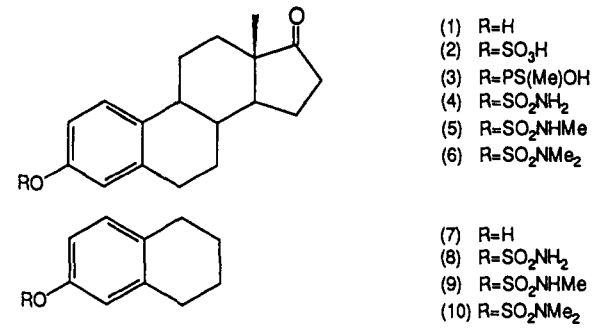

Figure 1. Structures of synthetic sulfamate analogues.

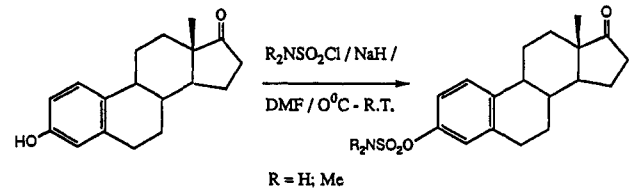

Figure 2. Synthesis of estrone sulfamates.

set of derivatives: sulfamates of both estrone (1) and tetrahydronaphth-2-ol (7) (Figure 1). The former, in particular, exhibit activity both as competitive and irreversible inhibitors and are much more potent than E1-3-MTP.

The sulfamoyl group has been widely utilized as an activity-modifying substituent in several different classes of drugs. For example, the nucleoside antibiotic, nucleocidin, contains a 5′-sulfamoyl group, and many defluorinated analogues containing this group have been examined, exhibiting antibacterial, antiviral, antiparasitic, and herbicidal activities. A few have been found to exhibit antitumor activity[13,14] and 5′-sulfamoylated purinyl carbocyclic nucleosides are potent cytotoxic agents.[15] Sulfamoyl derivatives of ribavirin exhibit antiparasitic activity.[16] *N*-Substituted steroidal sulfamates have been synthesized,[17–21] including sulfamates of estradiol,[19,22–24] to block metabolic conjugation, but their ability to inhibit estrone sulfatase has not been explored. In our search for sulfate group surrogates we synthesized estrone 3-*O*-sulfamate (4), *N*-monomethylsulfamate (5), and *N*,*N*-dimethylsulfamate (6) as well as the corresponding derivatives of tetrahydronaphth-2-ol (8–10). The latter were designed in order to examine the effect of removing part of the steroid skeleton on inhibition of estrone sulfatase.

All six compounds were prepared by first treating a solution of the appropriate alcohol in anhydrous dimethylformamide with sodium hydride (60% dispersion; 2 equiv) at 0 °C. Figure 2 illustrates the synthesis for estrone 3-*O*-sulfamate. (All new compounds were purified by silica gel flash column chromatography, were single spot pure by TLC, and exhibited satisfactory spectroscopic and microanalytical data.) After evolution of hydrogen had ceased, the appropriate sulfamoyl chloride (2 equiv) was added and the reaction mixture was allowed to warm to room temperature overnight. An aqueous workup, followed by chromatographic purification of the organic product, afforded the desired compounds in yields up to 70% [the lowest yields were obtained for the (monomethylamino)sulfamates 5 and 9]. Sulfamoyl chloride, required for the synthesis of 4 and 8, was prepared by reaction of chlorosulfonyl isocyanate with formic acid according to the method of Appel and Berger.[25] *N*-

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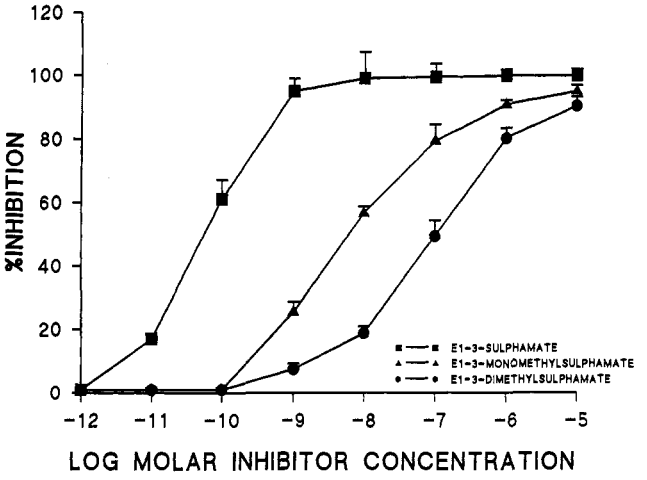

Figure 3. Dose–response curves for inhibition of estrone sulfatase in intact MCF-7 breast cancer cells by estrone 3-*O*-sulfamate (■), estrone 3-*O*-(*N*-methyl)sulfamate (▲), and estrone 3-*O*-(*N*,*N*-dimethyl)sulfamate (●). Assays were performed essentially as described previously.[10] Monolayers of MCF-7 cells in 25-cm^3 flasks were incubated for 20 h at 37 °C with [^{3}H]estrone sulfate (2 nM) and synthetic analogues at various concentrations. Estrone sulfatase activity was determined by measuring the total amount of ^{3}H-labeled estrone and estradiol formed. Sulfatase activity in untreated cells was 100–120 fmol/20 h/10^6 cells. Each point represent the mean ± sd of triplicate measurements.

Methylsulfamoyl chloride, used in the synthesis of **5** and **9**, was prepared from *N*-methylsulfamic acid by treatment with phosphorus pentachloride.[26] Commercially available *N*,*N*-dimethylsulfamoyl chloride was used to synthesize **6** and **10**.

Inhibition of estrone sulfatase activity by the sulfamates in intact MCF-7 breast cancer cells was examined using a physiological (2 nM) concentration of estrone sulfate as substrate. Concentration–response curves are illustrated for the steroid sulfamates in Figure 3. It was found that **4** inhibited estrone sulfatase activity by greater than 99% at 10, 1, and 0.1 μM, **5** exhibited an inhibition of 93% and 87% at 10 and 1 μM, respectively, and **6** showed an inhibition of 90%, 87%, and 79% at 10, 1, and 0.1 μM, respectively. These compounds were all more potent inhibitors of estrone sulfatase activity than E1-3-MTP (**3**), which showed inhibition of 96%, 74%, and 52% at 10, 1, and 0.1 μM, respectively,[10,12] but the extent of inhibition decreased with increasing methylation of the sulfamoylamino group. The IC_{50} for inhibition of estrone sulfatase by **4**, measured in a placental microsomal preparation (100 000-g pellet)[9] using a saturating substrate concentration (20 μM), was 80 nM. This again demonstrated the dramatic increase in potency of this compound compared to E1-3-MTP (**3**), since under the same conditions, the latter had an IC_{50} of 43 μM.[10,12]

The irreversible/reversible nature of enzyme inhibition by analogues **4**–**6** was also explored. Intact monolayers of MCF-7 cells were grown to 80% confluence and pretreated with medium, with or without analogues **4**–**6** (1 μM), for a 2-h period at 37 °C. The medium was then removed, and the monolayers were washed twice with PBS (5 mL) and assayed over a 20-h period for remaining sulfatase activity using [^{3}H]estrone sulfate. After 20 h, no sulfatase activity was detectable for **4**, while for **5** and **6** the sulfatase activity had recovered by greater than 50% and 80% of the control for **5** and **6**, respectively, but not for **4**. Additionally, preincubation of **4** or **6** with placental microsome preparations was carried out at 37 °C for 2 h

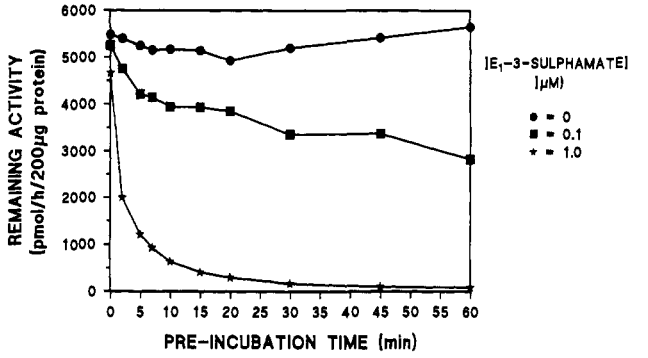

Figure 4. Time- and concentration-dependent inactivation of estrone sulfatase by estrone 3-*O*-sulfamate. Placental microsomes (200 μg of protein) were preincubated with **4** (0, ●; 0.1, ■; and 1 μM, *) for 0–60 min at 37 °C followed by incubation with dextran-charcoal for 10 min at 4 °C. Dextran-charcoal was sedimented by centrifugation, and portions of the supernatants were then incubated with [^{3}H]estrone sulfate (20 μM) for 1 h at 37 °C to assess remaining sulfatase activity. Duplicate experiments were run at each concentration, but assays for residual activity were taken at different times in each experiment.

followed by overnight dialysis at 4 °C. Reassay of estrone sulfatase activity revealed no recovery of activity with **4**, whereas for **6** activity was restored by 80%. Furthermore, placental microsomes were preincubated for 0–60 min with **4** (0.1 and 1.0 μM) and then treated with charcoal for 10 min at 4 °C to remove unbound inhibitor before assaying for remaining sulfatase activity. Estrone sulfatase activity was inactivated in a time- and dose-dependent manner (Figure 4).

The inability to detect estrone sulfatase activity when reassaying pretreated, washed MCF-7 cells suggest that **4** is an irreversible inhibitor while **5** and **6** are reversible inhibitors. Further evidence of the irreversible nature of the binding of **4**, but not **6**, to the estrone sulfatase enzyme was provided by the failure of activity to recover in placental microsomes after overnight dialysis with **4**, in contrast to the 80% recovery which occurred with **6**. Kinetic studies showed that **4** inactivates estrone sulfatase in a time- and dose-dependent manner, thus confirming that **4** is an irreversible inhibitor.

Upon examining the abilities of the tetrahydronaphth-2-ol derivatives **8**–**10** to inhibit estrone sulfatase activity in intact MCF-7 breast cancer cells, it was found that **8** inhibited estrone sulfatase activity by 97% and 47% at 10 and 1 μM, respectively; **9** showed an inhibition of 22% at 10 μM; and **10** exhibited no inhibition at either 10 or 1 μM. Again, it appeared that the extent of inhibition decreased with increasing methylation of the sulfamoyl group. However, since none of these compounds proved to be as potent as their corresponding estrone analogues, it can be concluded that the entire steroidal skeleton is required for optimal inhibition.

In conclusion, we have shown that estrone 3-*O*-sulfamates represent a new class of sulfatase inhibitor and are considerably more potent in MCF-7 breast cancer cells than E1-3-MTP (**3**), the best inhibitor to date. From the results obtained after reassaying pretreated, washed, MCF-7 cells, postdialysis microsomes and time- and dose-dependent inactivation of the enzyme, it is apparent that **4** is an irreversible inhibitor. The nature of the inhibition changes from irreversible to reversible on going from the unsubstituted estrone 3-*O*-sulfamate **4** to the disubstituted estrone 3-*O*-(*N*,*N*-dimethyl)sulfamate **6**. We do not yet understand the rationale for the activity of these sulfa-

mates, but it is conceivable that 4 causes irreversible sulfamoylation of estrone sulfatase. Clearly, estrone sulfamates are emerging as key lead compounds in the design of highly potent estrone sulfatase inhibitors, and such compounds may ultimately be of therapeutic utility.

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