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SYNTHESIS AND SULFATASE INHIBITORY ACTIVITIES OF (*E*)- AND (*Z*)-4-HYDROXYTAMOXIFEN SULFAMATES

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Abstract: We report the development of (*E*)- and (*Z*)-4-hydroxytamoxifen sulfamates as estrone sulfatase inhibitors, potential therapeutic agents for the treatment of breast cancer. Both compounds competitively inhibit estrone sulfatase isolated from rat liver with apparent K_i of 35.9 μM for (*E*)-4-hydroxytamoxifen sulfamate and an apparent K_i of > 500 μM for the (Z) isomer.

Estrogen levels in breast tumors of post-menopausal women are at least ten times higher than estrogen levels in plasma.[1,2] The high levels of estrogen in these tumors are presumably due to *in situ* formation of estrogen, possibly through conversion of estrone sulfate to estrone by the enzyme estrone sulfatase.[3,4] Therefore, inhibitors of estrone sulfatase are potential agents for the treatment of estrogen-dependent breast cancers. A number of estrone sulfatase inhibitors (both steroidal and nonsteroidal) have been developed.[5-19] Among all the estrone sulfatase inhibitors, estrone-3-*O*-sulfamate (EMATE) and its analogs are the most potent (Figure 1).[8,9] EMATE is classified as an active-site directed irreversible inhibitor.[13] Recently, nonsteroidal estrone sulfatase inhibitors were developed[13,14,18,19] based on the fact that EMATE was found to be estrogenic (Figure 1).[20] Both coumarin sulfamate and (*p*-*O*-sulfamoyl)-*N*-tetradecanoyl tyramine (DU-14) were reported to inactivate estrone sulfatase in an active-site directed manner.[13,18] As shown in Figure 1, it can be concluded that B, C, and D rings of the steroid nucleus is not required for binding to estrone sulfatase and the common functionality for sulfatase inactivation is a phenylsulfamoyl group. Thus, it occurs to us that a potent antiestrogen such as 4-hydroxytamoxifen can be easily converted to the respective sulfamate analog and becomes potential dual inhibitor (inhibitor with sulfatase inhibitor activity and antiestrogenic activity).

The antiestrogen nuclei we select are (*Z*)-4-hydroxytamoxifen and its *cis* analog (*E*)-hydroxytamoxifen. (*Z*)-4-hydroxytamoxifen is the active metabolite of tamoxifen in vivo. We report herein the synthesis and estrone-sulfatase inhibitory activities of (*E*)- and (*Z*)-4-hydroxytamoxifen sulfamates (**1** and **2**) (Figure 1).

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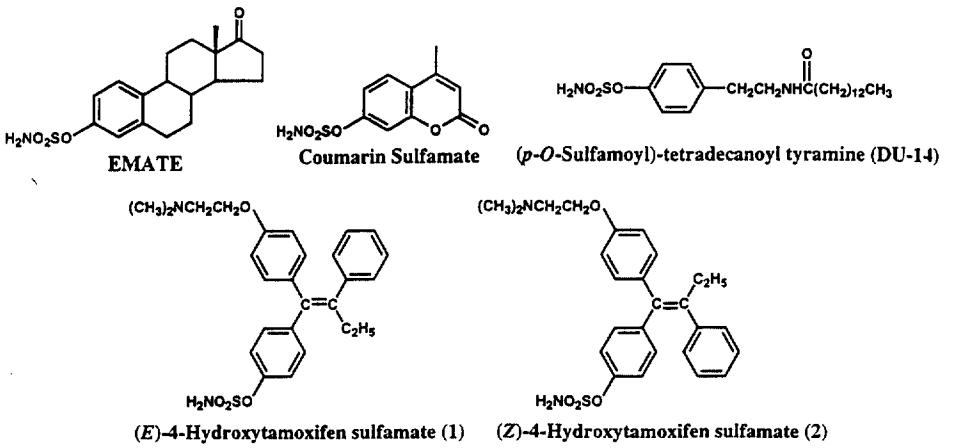
Figure 1. Structures of estrone sulfatase inhibitors and (*E*)- and (*Z*)-4-hydroxytamoxifen sulfamates



Inhibitors **1** and **2** were synthesized by sulfamoylation[14] of the (*Z*)- and (*E*)-4-hydroxytamoxifens in 93 and 99% yields, respectively (Figure 2). Both (*Z*)- and (*E*)-4-hydroxytamoxifens were stereospecifically synthesized according to the literature procedure,[21] which was based on McMurry reaction[22] as the key step. In the case of the synthesis of (*Z*)-4-hydroxytamoxifen, the McMurry reaction involved the coupling of monopivaloated benzophenone **3** with propiophenone to form compound **4** with *E*/*Z* ratio of 14/1. The *E*/*Z* ratio of > 100/1 could be obtained by trituration from methanol (Figure 3).[21] However, depivaloation at the later step using CH_3Li afforded modest yield (60%). We have attempted to replace the pivaloyl protecting group in the McMurry reaction with groups such as benzyl ($PhCH_2$-), triphenylmethyl (Ph_3C-) and carbobenzoxy (Cbz - $PhCH_2OCO$-) groups (Figure 3). We chose these protecting groups because they can be cleaved in high yield and also under neutral condition so can avoid possible isomerization.[23] For example, in our nonstereospecific synthesis of (*Z*)- and (*E*)-4-hydroxytamoxifens, the benzyl protecting group can be cleaved in virtually quantitative yield using hydrogenation without reducing the double bond (Figure 4). Unfortunately, the McMurry reaction in Figure 3 using the selected protecting groups did not give isomers with improved *E*/*Z* ratio (10/1 or less) when compared to the pivaloyl group.

Figure 2. Synthesis of (*E*)- and (*Z*)-4-hydroxytamoxifen sulfamates

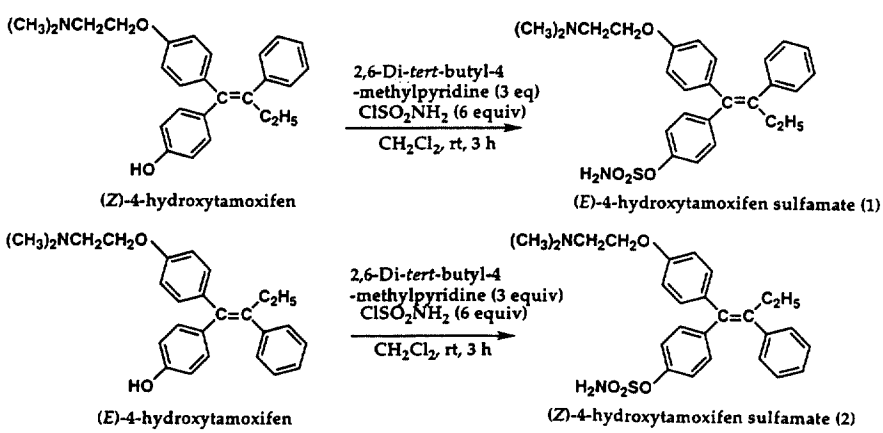


Figure 3. McMurry reaction using different monosubstituted benzophenones

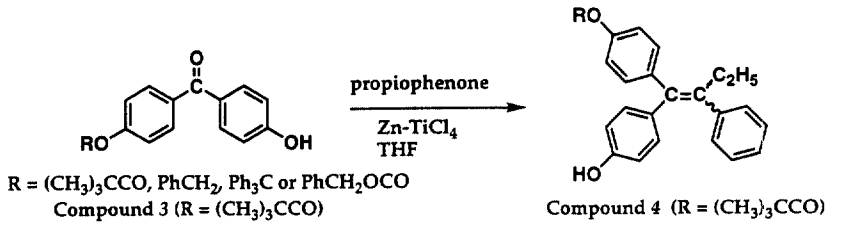
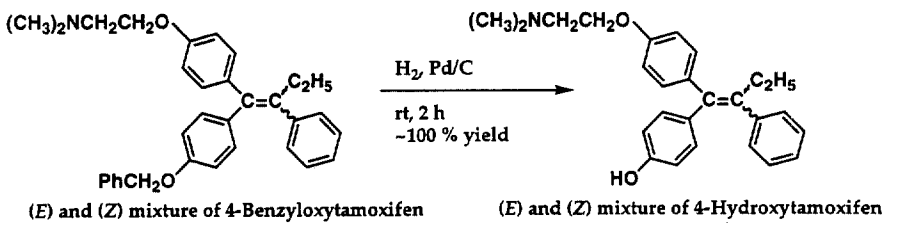
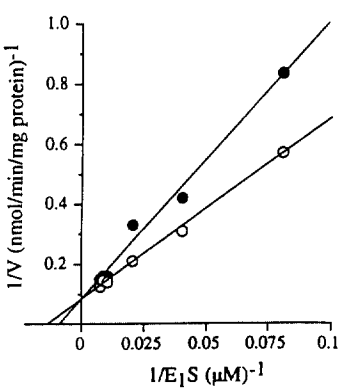


Figure 4. Debenzylation of 4-benzyloxytamoxifen through hydrogenation



Inhibitors **1** and **2** were evaluated in vitro by enzyme kinetic studies using rat liver microsomes as the source of estrone sulfatase. (*E*)-Hydroxytamoxifen sulfamate competitively inhibited estrone sulfatase and exhibited an apparent K_i of 35.9 ± 4.4 μM (Figure 5). It had a higher affinity than the substrate estrone sulfate, since the K_m of the substrate is 90.2 ± 8.0 μM. Conversely, (*Z*)-4-hydroxytamoxifen sulfamate was a much weaker inhibitor with an apparent K_i of > 500 μM. Enzyme inactivation studies are in progress. Further evaluation of these analogs in cell culture systems and in vivo will provide additional information on the efficacy of these inhibitors for the treatment of estrogen-dependent cancers.

Figure 5. Lineweaver-Burk plot showing inhibition of rat liver microsome estrone sulfatase activity by (*E*)-4-hydroxytamoxifen sulfamate **1**. Rat liver microsomes (25 μg) were incubated with E_1S (12.5-150 μM radioinert E_1S containing 150,000 dpm ^3H-E_1S) in the absence (o) or presence (•) of the inhibitor (42 μM) in a 50 mM Tris-HCl (pH 7.5) buffer (500 μL total volume). Reaction was incubated 20 min at 37° C, then stopped by addition of 0.1 N NaOH. Unconjugated tritiated steroids (E_1 and E_2) were extracted using toluene, and radioactivity was measured by liquid scintillation.



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