

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

Guo-Hua Chu,<sup>a</sup> Amy Peters,<sup>b</sup> Kyle W. Selcer,<sup>b</sup> and Pui-Kai Li,<sup>a\*</sup>

<sup>a</sup>*Department of Medicinal Chemistry and Pharmaceuticals, Mylan School of Pharmacy and*

<sup>b</sup>*Department of Biology, Bayer School of Natural and Environmental Sciences,*

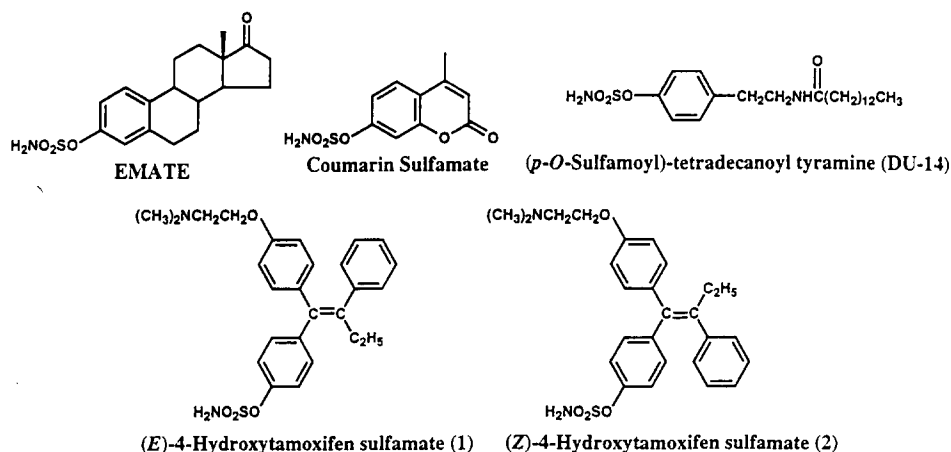
*Duquesne University, Pittsburgh, PA 15282, U.S.A.*

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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  $\mu\text{M}$  for (*E*)-4-hydroxytamoxifen sulfamate and an apparent  $K_i$  of > 500  $\mu\text{M}$  for the (*Z*) isomer. © 1999 Elsevier Science Ltd. All rights reserved.

Estrogen levels in breast tumors of post-menopausal women are at least ten times higher than estrogen levels in plasma.<sup>1,2</sup> 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.<sup>3,4</sup> 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.<sup>5–19</sup> Among all the estrone sulfatase inhibitors, estrone-3-*O*-sulfamate (EMATE) and its analogs are the most potent (Figure 1).<sup>8,9</sup> EMATE is classified as an active-site directed irreversible inhibitor.<sup>13</sup> Recently, nonsteroidal estrone sulfatase inhibitors were developed<sup>13,14,18,19</sup> based on the fact that EMATE was found to be estrogenic (Figure 1).<sup>20</sup> Both coumarin sulfamate and (*p*-*O*-sulfamoyl)-*N*-tetradecanoyl tyramine (DU-14) were reported to inactivate estrone sulfatase in an active-site directed manner.<sup>13,18</sup> 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).

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

Inhibitors **1** and **2** were synthesized by sulfamoylation<sup>14</sup> 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,<sup>21</sup> which was based on McMurry reaction<sup>22</sup> 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).<sup>21</sup> However, depivaloation at the later step using  $\text{CH}_3\text{Li}$  afforded modest yield (60%). We have attempted to replace the pivaloyl protecting group in the McMurry reaction with groups such as benzyl ( $\text{PhCH}_2$ -), triphenylmethyl ( $\text{Ph}_3\text{C}$ -) and carbobenzoxy (Cbz -  $\text{PhCH}_2\text{OCO}$ -) 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.<sup>23</sup> 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.

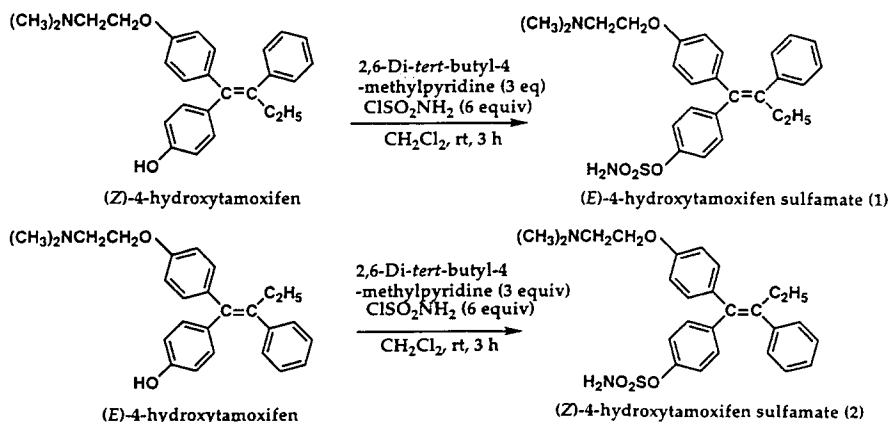
Figure 2. Synthesis of (*E*)- and (*Z*)-4-hydroxytamoxifen sulfamates

Figure 3. McMurry reaction using different monosubstituted benzophenones

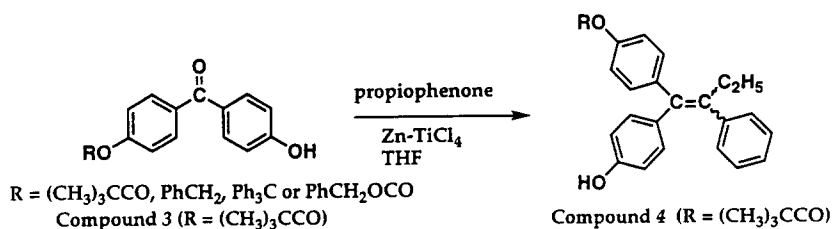
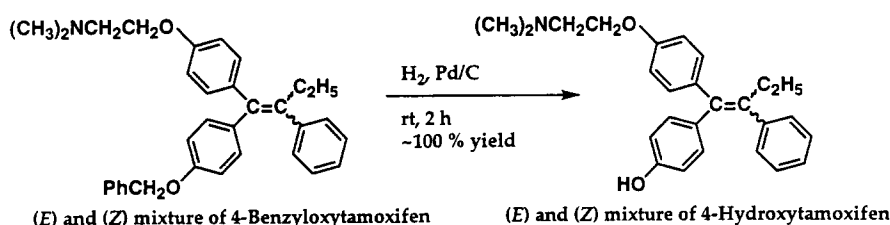
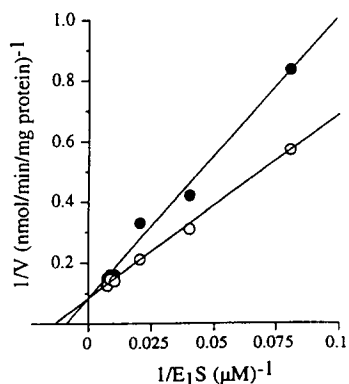


Figure 4. Debenzylation of 4-benzyloxytamoxifen through hydrogenation



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  $35.9 \pm 4.4 \mu\text{M}$  (Figure 5). It had a higher affinity than the substrate estrone sulfate, since the  $K_m$  of the substrate is  $90.2 \pm 8.0 \mu\text{M}$ . Conversely, (*Z*)-4-hydroxytamoxifen sulfamate was a much weaker inhibitor with an apparent  $K_i$  of  $> 500 \mu\text{M}$ . 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  $\mu\text{g}$ ) were incubated with  $\text{E}_1\text{S}$  (12.5–150  $\mu\text{M}$  radioinert  $\text{E}_1\text{S}$  containing 150,000 dpm  $^3\text{H-E}_1\text{S}$ ) in the absence (○) or presence (●) of the inhibitor (42  $\mu\text{M}$ ) in a 50 mM Tris-HCl (pH 7.5) buffer (500  $\mu\text{L}$  total volume). Reaction was incubated 20 min at  $37^\circ\text{C}$ , then stopped by addition of 0.1 N NaOH. Unconjugated tritiated steroids ( $\text{E}_1$  and  $\text{E}_2$ ) were extracted using toluene, and radioactivity was measured by liquid scintillation.



## References and Notes

1. Noel, C. T.; Reed, M. J.; Jacobs, H. S.; James, V. H. T. *J. Steroid Biochem.* **1981**, *14*, 1101.
2. Samojlik, E.; Santen, R. J.; Worgul, T. J. *Steroids* **1982**, *39*, 497.
3. Santner, S. J.; Feil, P. D.; Santen, R. J. *J. Clin. Endocrinol. Metab.* **1984**, *59*, 29.
4. Pasqualini, J. R.; Chetrite, G.; Nguyen, B. L.; Maloche, C.; Delalonde, L.; Talbi, M.; Feinstein, M. C.; Blacker, C.; Botella, J.; Paris, J. *J. Steroid Biochem. Molec. Biol.* **1995**, *59*, 407.
5. Duncan, L.; Purohit, A.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Cancer Res.* **1993**, *53*, 298.
6. Howarth, N. M.; Cooper, G.; Purohit, A.; Duncan, L.; Reed, M. J.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 313.
7. Reed, M. J.; Purohit, A. *Rev. Endocrine-Related Cancer* **1993**, *45*, 51.
8. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 219.
9. Purohit, A.; Vernon, K. A.; Hummelinck, A. E.; Woo, L. W.; Hejaz, H. A.; Potter, B. V.; Reed, M. J. *J. Steroid Biochem. Molec. Biol.* **1998**, *64*, 269.
10. Li, P. K.; Pillai, R.; Young, B. L.; Bender, W. H.; Martino, D. M.; Lin, F. T. *Steroids* **1993**, *58*, 106.
11. Dibbelt, L.; Li, P. K.; Pillai, R.; Knuppen, R. *J. Steroid Biochem. Molec. Biol.* **1995**, *52*, 281.
12. Li, P. K.; Pillai, R.; Dibbelt, L. *Steroids* **1995**, *60*, 299.
13. Selcer, K. W.; Jagannathan, S.; Rhodes, M. E.; Li, P. K. *J. Steroid Biochem. Molec. Biol.* **1996**, *59*, 83.
14. Li, P. K.; Milano, S.; Kluth, L.; Rhodes, M. E. *J. Steroid Biochem. Molec. Biol.* **1996**, *59*, 41.
15. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508.
16. Purohit, A.; Williams, G. J.; Roberts, C. J.; Potter, B. V. L.; Reed, M. J. *Int. J. Cancer* **1995**, *63*, 106.
17. Woo, L. W.; Lightowler, M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Steroid Biochem. & Mol. Biol.* **1996**, *57*, 79.
18. Woo, L. W.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1996**, *39*, 1349.
19. Woo, L. W.; Howarth, N. M.; Purohit, A.; Hejaz, H. A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, *41*, 1068.
20. Elger, W.; Schwarz, S.; Hedden, A.; Reddersen, G.; Schneider, B. *J. Steroid Biochem. Molec. Biol.* **1995**, *55*, 396.
21. Gauthier, S.; Mailhot, J.; Labrie, F. *J. Org. Chem.* **1996**, *61*, 3890.
22. McMurry, J. E. *Chem. Rev.* **1989**, *89*, 1513.
23. Shani, J.; Gazit, A.; Livshitz, T.; Biran, S. *J. Med. Chem.* **1985**, *28*, 1504.