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SYNTHESIS AND ESTROGEN RECEPTOR BINDING AFFINITIES OF THE MAJOR HUMAN METABOLITES OF RALOXIFENE (LY139481)

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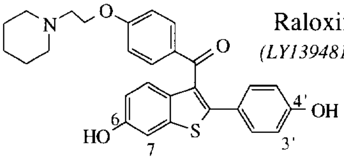
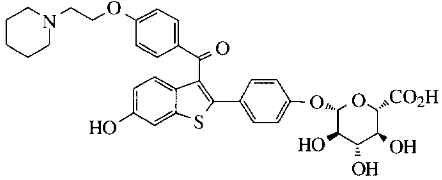
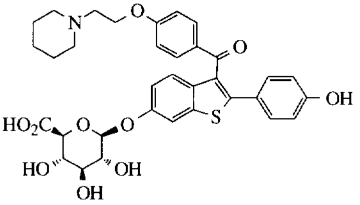
Abstract: Glucuronide conjugates **1** and **2**, the major metabolites of raloxifene, have been prepared and their molecular interactions with the estrogen receptor determined. © 1997 Elsevier Science Ltd.

The benzothiophene raloxifene,[1] currently in advanced clinical trials for the prevention and treatment of osteoporosis, displays beneficial bone and cardiovascular effects in post-menopausal women without causing concomitant uterine hypertrophy.[2] This unique clinical profile is supported by numerous preclinical studies[3] on bone, lipids, breast, or uterus and has led to the classification of raloxifene as a selective estrogen receptor modulator (SERM).[4,5] Despite the abundance of data demonstrating tissue selective pharmacology for raloxifene, the molecular origin(s) responsible for this phenomenon remain largely unknown. Katzenellenbogen,[6] McDonnell,[7] and others,[8] have proposed that individual estrogens and anti-estrogens may interact uniquely with the estrogen receptor and/or associated effector sites. In this context, discreet receptor interactions ultimately result in differential gene activation and varying pharmacological profiles. Alternatively, metabolic and pharmacokinetic parameters may play a significant role in dictating tissue selectivity. For example, metabolism of raloxifene to its glucuronide conjugates (**1** and **2**) occurs to the extent that very little circulating free raloxifene exists in the serum of humans after oral administration.[9] Given the magnitude of phenolic glucuronidation, and the relative abundance of the resulting conjugates in serum, we have investigated the potential role these metabolites play at the estrogen receptor level. Herein, we report (a) the first syntheses of the major human metabolites of raloxifene, including an isotopically labeled form, and (b) the relative affinities of these glucuronide conjugates for the estrogen receptor.

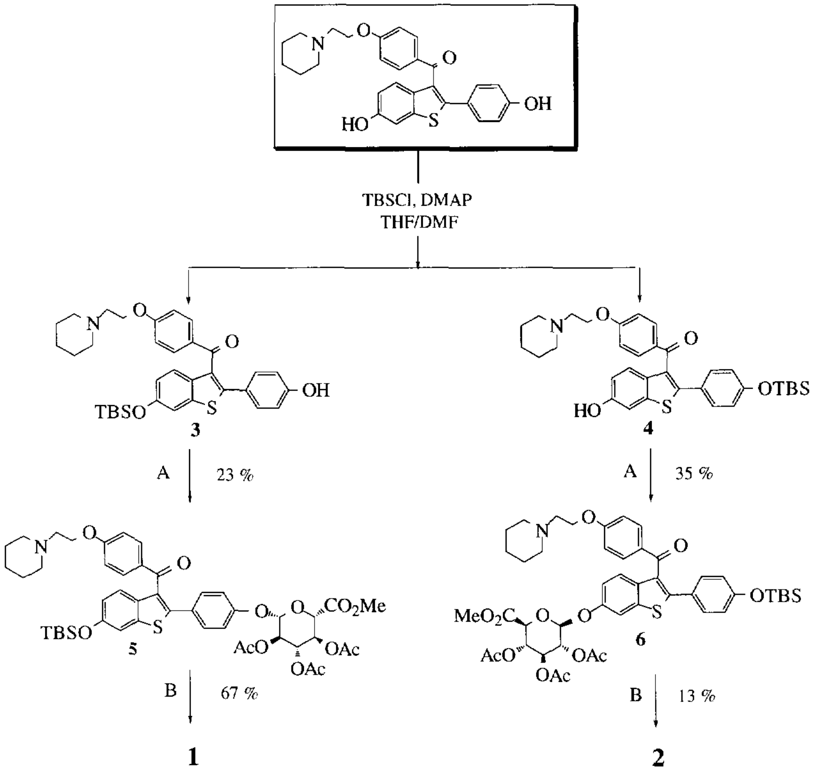

Raloxifene
(LY139481 HCl)


Raloxifene-4'-β-glucuronide (**1**)


Raloxifene-6-β-glucuronide (**2**)

Synthesis of the 4'-, and 6-β-glucuronides of raloxifene (**1** and **2**, respectively) required *O*-glycosylation of the corresponding 4' and 6 phenolic substrates. As shown in Scheme 1, differentiation of these two phenol moieties was accomplished by reaction of raloxifene with *tert*-butyldimethylsilyl chloride which gives a chromatographically separable mixture of **3** and **4**. The regiochemistry of the silylation process was tentatively assigned by the >0.5 ppm changes in chemical shift observed in the *ortho* protons flanking each of the newly formed silyl ethers. Lewis acid mediated coupling[10] of phenol **3** or **4** with methyl-1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate[11] provides a single product in each case with the desired β-stereochemistry at the anomeric center as indicated by the 7.4 Hz *trans* diaxial coupling constant observed between the 1'-H and 2'-H in each of the glycosydic moieties. Subsequent removal of the ester protecting groups proved problematic. For example, treatment of **5** or **6** with NaOH resulted in immediate cleavage of the sugar moiety to give raloxifene. Similar fragmentation was observed under a variety of standard saponification conditions including barium hydroxide, magnesium hydroxide, and NH_3/methanol. After considerable experimentation, we found that heating **5** or **6**

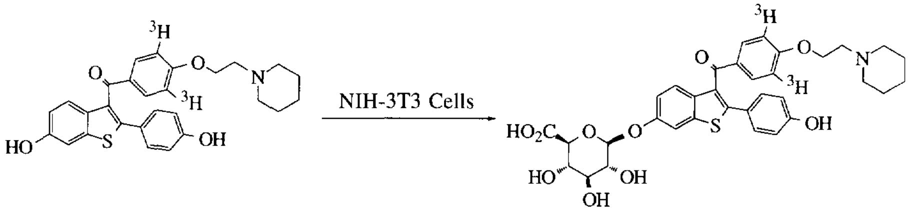
Scheme 1



Reagents and Conditions: A. Methyl-1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate, $BF_3.OEt_3$, CH_2Cl_2. B. (1) LiOH, dioxane, (2) tetrabutylammonium fluoride, THF.

with lithium hydroxide in dioxane to 60 °C, followed by cooling of the reaction mixture and treatment with tetrabutylammonium fluoride, gives the respective glucuronides, **1** and **2**, in moderate to poor yields. The initial regiochemical assignments made for **3** and **4** were confirmed by difference NOE studies in **1** and **2** showing enhancements between the anomeric proton and the appropriate benzothiophene proton(s), i.e., H-7 for **2**; 3'-H and 5'-H for **1**. In addition, a 10 Hz coupling *trans* coupling constant between 5'-H and 4'-H of the glycoside of **1** indicates the carboxylate moiety remains equatorially disposed even under the relatively harsh saponification conditions. Lastly, correlation of **1** and **2** with authentic samples, previously isolated and characterized from microsomal sources, shows each synthetic metabolite to be identical with its natural counterpart by NMR, mass spectra, and reverse phase HPLC co-injection.

With sufficient quantities of **1** and **2** in hand for evaluation in receptor binding and other assays, we subsequently investigated the preparation of a radiolabeled form of **2** for in vivo tissue distribution studies. Because of the low overall chemical yields incurred in the synthesis of **2** (Scheme 1) and the ready availability of tritiated raloxifene starting material (**7**)[12] we elected to pursue a whole cell preparation of ^{3}H-**2**. Glucuronidation is well-documented in a variety of in vitro systems, including microsomal preparations,[13] intestinal epithelial cells,[14] and hepatocytes,[15] many of which result in selective O-glycoside formation.[9a,13] Thus, a panel of cell lines (MC3T3-E1, NIH-3T3, MG-63, Hep-G2, and MCF-7) were screened for their ability to glucuronidate raloxifene. Of these, only NIH-3T3 mouse fibroblast-like cells efficiently convert raloxifene to glucuronide conjugate. Incubation of **7** (30.1 Ci/mmol specific activity, radiopurity of >99 % by HPLC) with NIH-3T3 cells for 16 h at 37 °C followed by lyophilization, extraction, and purification of the cell medium provides a single product in 98.6 % radiochemical purity. Gratifyingly, this whole-cell preparation results in complete regio–, and stereoselective formation of tritiated metabolite **8**, as determined by correlation with authentic samples of unlabelled standard **2**, as well as chemical conversion to aglycone with β-glucuronidase. While stereoselective formation of *O*-glycosides is well precedented,[13] it is not entirely clear why regioselective formation of the 6-glucuronide occurs in this cell line. However, species defined metabolism has been observed with raloxifene, and the regioisomer obtained is consistent with the major metabolite detected in rodents.[9a]



7 **8**

Biological Evaluation. The tissue selective pharmacology of raloxifene is presumed to be mediated at the molecular level by it's interaction with the estrogen receptor.[16] Thus, the ability of metabolites **1** and **2** to compete with ^{3}H-17β-estradiol for receptor binding was determined in cell lysates derived from a human MCF-7 breast cancer cell line. In this assay, increasing concentrations of either metabolite are significantly less potent than raloxifene in displacing labeled ligand. As shown in Table 1, the relative binding affinity (RBA) for **1** and **2** is < 0.07 and < 0.09, respectively, which represents an order of magnitude decrease when compared to

Table 1. Relative estrogen receptor binding affinities for raloxifene, **1**, **2**, and 17β-estradiol.

cmpd	*RBA*[a]
raloxifene	0.20
1	< 0.07
2	< 0.09
17β-estradiol	1.0

[a] RBA = IC_{50} 17β-estradiol/ IC_{50} cmpd

raloxifene (RBA = 0.20). This data suggests that the tissue effects observed for raloxifene are not mediated by high affinity interactions of these metabolites with the estrogen receptor. These metabolites also demonstrate significantly diminshed functional activity when compared to raloxifene in a variety of estrogen-dependent cell-based assays.[17] Our continuing studies on raloxifene's tissue selectivity focus on examining the pharmacokinetic distribution of radiolabeled metabolite **8** in our in vivo rodent models.

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References and Notes

1. Jones, C. D.; Hevnikar, M. G.; Pike, A. J.; Peters, M. K.; Black, L. J.; Thompson, A. R.; Falcone, J. F.; Clemens, J. A. *J. Med. Chem.* **1984**, *27*, 1057.

2. Draper, M. W.; Flowers, D. E.; Huster, W. J.; Neild, J. A.; Harper, J. E.; Arnaud, C. *J. Bone Min. Res.* **1996**, *11*, 835.

3. >200 publications and abstracts have appeared on various pharmacological aspects of raloxifene since 1981.

4. Representative publications: *Bone, lipids, and uterus:* Sato, M.; Bryant, H. U.; Iverson, P.;Helterbrand, J.; Smietana, F.; Bemis, K.; Higgs, R.; Turner, C. H.; Owan, I.; Takano, Y.; Burr, B. B. *J. Pharm. Exp. Therap.* **1996**, *279*, 298. Black, L. J.; Sato, M.; Rowley, E. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffman, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, J. D.; Bryant, H. U. *J. Clin. Invest.* **93** (1994) 63. *Breast:* Clemens, J. A.; Bennet, D. R.; Black, L. J.; Jones, C. D. *Life Sci.* **1983** *322*, 869. Gottardis, M. M.; Jordan, V. C. *Cancer Res.* **1987** *47*, 4020.

5. For a review see Grese, T. A.; Dodge, J. A. *Ann. Rep. Med. Chem.* **1996**, *31*, 181.

6. Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. *Mol. Endocrinol.* **1996**,*10*, 119.

7. McDonnell, D. P.; Clemm, D. L.; Hermann, T.; Goldman, M. E.; Pike, J. W. *Mol. Endocrinol.* **1995**, *9* 659.

8. Willson, T. W.; Henke, B. R.; Momtahen, T. M.; Charifson, P. S.; Batchelor, K. W.; Lubahn, D. B.; Morre, L. B.; Oliver, B. B.; Sauls, H. R.; Triantafillou, J. A.; Wolfe, S. G.; Baer, P. G. *J. Med. Chem.* **1994**, *37*, 1550.

9. (a) Lindstrom, T. D.; Whitaker, N. G.; Whitaker, G. W. *Xenobiotica* **1984**, *11*, 841. (b) Forgue, S. T.; Rudy, A. C.; Knadler, M. P.; Basson, R. P.; Nelson, J. E.; Henry, D. P.;Allerhèiligen, S. R. *Pharmaceutical Res.* **1996**, *13*, S-429. (c) Ni, L.; Allerheiligen, S. R.; Basson, R; Knadler, M. P.; Latz, J.; Geiser, J.; Lantz, R.; Rash, J.; Henry, D. P. *Pharmaceutical Res.* **1996**, *13*, S-430.

10. Mabic, S.; Benezra, C.; Lepoittevin J-P. *Tetrahedron Lett.* **1993**, *34*, 4531.

11. Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310.

12. Dodge, J. A.; Stocksdale, M. G.; Jones, C. D. *J. Labelled. Cmpds. Radiopharm.* **1994**, *XXXVI*, 43.

13. Dahl-Puustinen, M. L.; Dumont, E; Bertilsson, L. *Drug. Metab. Dispos.* **1989**, *17*, 433.

14. Singh. J.; Dubey, R. K.; Atal, C. K. *J. Pharmacol. Exp. Ther.* **1986**, *236*, 488.

15. Schrenk, D.; Bock K. W. *Drug. Metab. Dispos.* **1990**, *18*, 720.

16. Yang, N. N.; Venugopalan, M.; Hardikar, S.; Glasebrook, A. *Science* **1996**, *273*, 1222.

17. Dodge, J. A.; Lugar, C. W.; Cho, S.; Short, L. L.; Sato, M.; Yang, N. N.; Spangle, L. A.; Martin, M. J.; Phillips, L.; Glasebrook, A. L.; Osborne, J. J.; Frolik, C. A.; Bryant, H. U. *J. Steroid Biochem. Molec. Biol.* **1997**, in press. This manuscript contains full experimental details for the preparation of **1**, **2**, and **8**, including structural characterization.

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