



VERSATILE RALOXIFENE TRIFLATES

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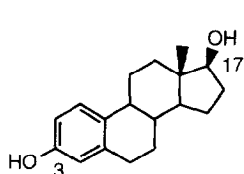
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Abstract: Methodology has been employed that permits the differentiation of the phenols of raloxifene. Transition metal mediated transformations of raloxifene triflates have subsequently provided a number of analogs that were evaluated further in two in vitro models predictive of estrogen receptor mediated biological activity. © 1997 Elsevier Science Ltd.

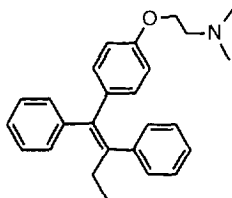
The effect of naturally occurring estrogens, such as 17 β -estradiol, on numerous tissues has long been recognized. In postmenopausal women, specifically, estrogen production is dramatically diminished, and the loss of its protective effects on the skeleton is a major health concern.^{1a} Decreased estrogen levels have also been implicated in other pathologies, such as depression and schizophrenia,^{1b} cardiovascular disease,^{1c} and Alzheimer's disease.^{1c}

Hormone replacement therapy can restore estrogen levels to a protective state, but reproductive cancer risks and side effects preclude widespread acceptance.^{1a-c} Nonsteroidal "antiestrogens" like tamoxifen that act through the estrogen receptor (ER) have been developed to antagonize the negative effects of estrogens in, for example, breast tissue.^{1d-g} Of particular interest are "antiestrogens" that exhibit antagonist effects in reproductive tissues but mimic estrogen in the skeletal and cardiovascular systems. Raloxifene **1** has been identified as a nonsteroidal, selective estrogen receptor modulator (SERM), and is currently under evaluation for the treatment and prevention of osteoporosis.^{2a-e}

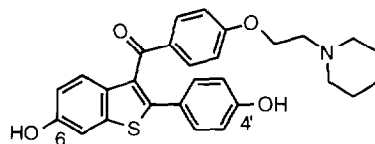
Figure 1



17- β -estradiol



tamoxifen



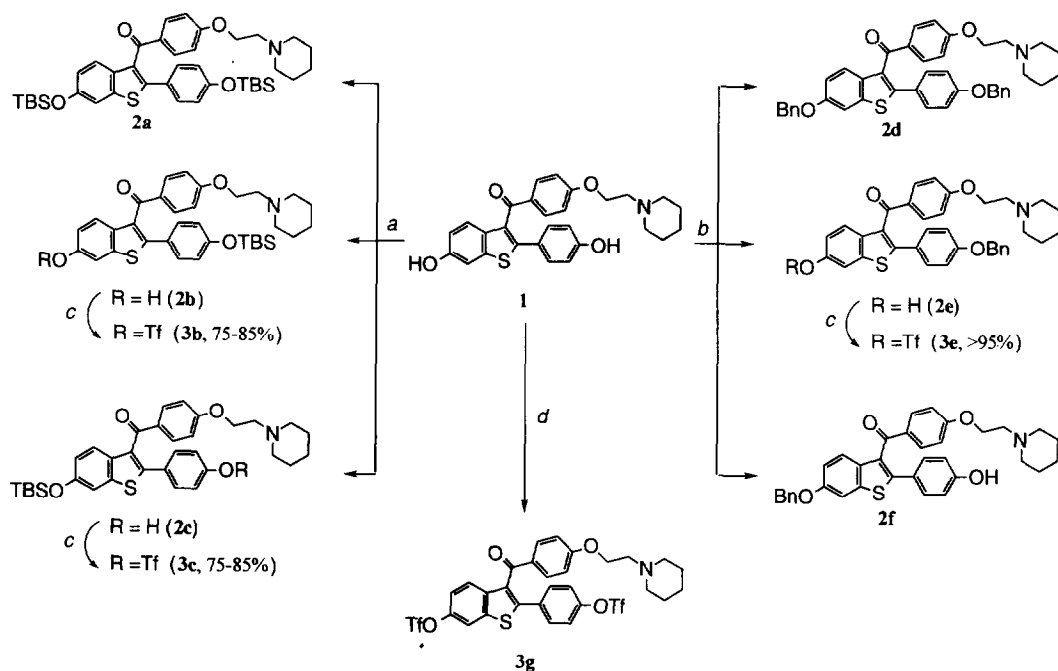
raloxifene, **1**

The importance of the hydroxyl groups of 17 β -estradiol, specifically the phenol at position-3, in ER binding has been detailed.^{1f,g} It is probable that the phenolic moieties present in raloxifene mimic the hydroxyl functionalities of 17 β -estradiol, as has been described for other ER modulators.^{1d-g} In an attempt to clarify the role of the individual phenols of raloxifene, we have employed methodology that differentially transforms these residues. The resulting raloxifene analogs were then examined in two *in vitro* assays predictive of estrogen receptor mediated biological activity. Specifically, agents were assayed for their ability to bind to the estrogen receptor and to inhibit estrogen-stimulated proliferation of a mammary tumor cell line.

Chemistry

A variety of raloxifene analogs substituted at the 6- and 4'-phenolic positions were synthesized via transition metal (Pd and Ni) catalyzed reactions of substituted raloxifene triflates, as detailed below.

Scheme 1. Preparation of Raloxifene Triflates



(a) TBSCl, DMAP, THF, DMF, chromatography, ~20% each; (b) BnCl, NaH, DMF, chromatography, ~20% each;

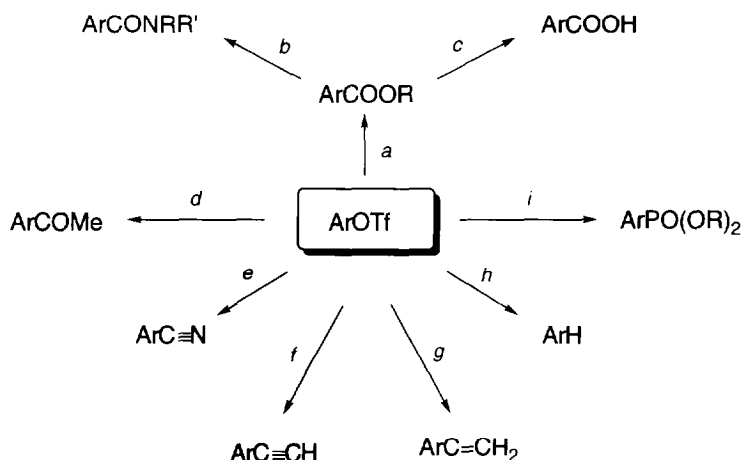
(c) Tf₂NPh, Et₃N, CH₂Cl₂; (d) Tf₂O, Et₃N, ClCH₂CH₂Cl, 51%

As summarized in Scheme 1, raloxifene was differentially protected by treatment with TBSCl and DMAP to provide a 1:1:1 mixture of chromatographically separable silyl ethers 2a-c.³ This procedure was equally

effective using benzyl chloride and NaH to afford a similar mixture of benzyl ethers **2d-f**. Triflates **3b-c, e** and **g** were then prepared by treating raloxifene phenol derivatives with *N*-phenyltrifluoromethanesulfonimide or TiF_2O .⁴

Raloxifene triflates **3** were then converted into various analogs, as illustrated in Figure 2. Esters **4a-b**, **5a-c**, and **6a-c** (see Table) were prepared by palladium mediated carbonylation, with yields ranging from 27% to >95%.⁵ Partial deprotection of silyl ethers occurred in situ with both congeners, but reprotection of the exposed phenol group was easily effected for further transformation. Utilization of a benzyl ether at position-4' provided robust protection throughout the carbonylation (and subsequent) steps.

Figure 2. Raloxifene Triflate Derivatives



- (a) $\text{Pd}(\text{OAc})_2$, bis-diphenylphosphinopropane (dppp), Et_3N , DMF, ROH, CO, 27-95%; (b) $\text{Me}_3\text{AlNRR}'\text{HCl}$, PhCH_3 or NH_3 , MeOH or NaNH_2 , THF, 21-72%; (c) LiOH, THF or EtOH, 30-51%; (d) $\text{Pd}(\text{OAc})_2$, dppp, Et_3N , DMF, butylvinyl ether, 41-50%; (e) $\text{Ni}(\text{dppp})\text{Cl}_2$, KCN, Zn, MeCN, <15%; (f) $\text{Pd}(\text{OAc})_2$, dppp, Et_3N , DMF, TMS-C \equiv CH, 22-45%; (g) $\text{Pd}(\text{OAc})_2$, dppp, Et_3N , DMF, vinyl acetate, <7%; (h) $\text{Pd}(\text{OAc})_2$, dppp, Et_3N , DMF, HCO_2H , 60-71%; (i) $\text{Pd}(\text{PPh}_3)_4$, $\text{HPO}(\text{OEt})_2$, MeCN, 31-86%

Note Yields include desilylation (where appropriate) using TBAF/ CH_2Cl_2 or aq HCl/THF

Methyl esters **4a** and **5a** were derivatized further to provide additional analogs. Amides **4d-f** and **5e** were prepared primarily using the aminolysis procedure of Weinreb in 21-72% yield.⁶ Carboxylic acids **4c** and **5d** were obtained by saponification with LiOH in 30-51% yield. Conversion of the phenols of derivatives **4a** and **5a** into the respective triflates, followed by palladium mediated reduction with 95% HCOOH , afforded the hydro analogs **6b,c** in 60-71% yield.⁷

Other analogs were also available via these versatile aryl triflates. Monotriflates **3b,c** were converted under palladium catalysis into the acetyl⁸ **4g**, **5f** and diethylphosphonyl⁹ **4h**, **5g** derivatives, with overall yields ranging from 31-86%. The 4'-nitrile congener **4k** was prepared in modest yield (<15%) from triflate **3b**,¹⁰ and reaction of 4'-monotriflate **3b** with vinyl acetate afforded the 4'-vinyl derivative **4j** in low yield (<7%).¹¹ Interestingly, the attempted nitrile and vinyl transformations at position-6 using triflate **3c** failed uniformly. Finally, treatment of raloxifene monotriflates **3b,c** with TMS-C \equiv CH afforded the respective ethynyl analogs **4i** and **5h** in 22-45% yield.¹²

Biology¹³

Compounds were evaluated to determine ER binding affinity in an MCF-7 cell lysate through competitive displacement of tritiated 17 β -estradiol. Relative binding affinities are presented (RBA) as an average of at least two (2) determinations with an accuracy of $\pm 10\%$. Antagonist effects in breast tissue were assayed by inhibition of estrogen stimulated MCF-7 cell proliferation. Data are presented (IC₅₀) as the dose required to give 50% inhibition of a maximally effective dose (10^{-11} M) of 17 β -estradiol. Values represent an average of at least three (3) determinations with an accuracy of $\pm 10\%$. These data are summarized in the Table.

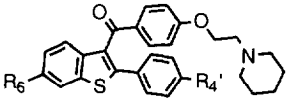
It should be noted that all compounds substituted at position-6 and/or -4' of raloxifene showed a marked decrease in binding and MCF-7 antiproliferative activity versus raloxifene itself, although most demonstrated improved inhibition of proliferation versus tamoxifen. In general, replacement of the 4'-phenol was tolerated marginally better than the comparable transformation at position-6, suggesting that the 6-phenol of raloxifene may mimic the 3-phenol of 17 β -estradiol in ER binding. Additionally, it appears that analogs bearing smaller groups at position-4' (**4g** and **4i-k**) exhibit better binding and antiproliferative activity overall, implying steric constraints to ER binding. Replacement of both phenolic moieties of raloxifene effectively abated all binding and proliferation antagonism, providing further evidence for the requirement of these functional groups for in vitro ER activity.

Conclusion

The monotriflate route to raloxifene analogs is an expedient method for the synthesis of compounds that would be difficult to make by other routes. The availability of the starting material and the flexibility of these intermediates has provided unique opportunities for the timely preparation of a wide variety of derivatives.

While the binding data for these raloxifene analogs indicate decreased affinity for the ER, the antiproliferative data suggest these congeners merit further consideration. Additionally, the propensity of the phenolic residues of raloxifene to undergo glucuronidation is well established.¹⁴ In this light, the potential in vivo activity of these raloxifene analogs is intriguing. It is conceivable that reducing the metabolic liability incurred by a free phenol would improve bioavailability. Further results in this area will be reported in due course.¹⁵

Table. Binding and Antiproliferative Data for Raloxifene Derivatives

			[³ H]-Estradiol RBA	Inhibition of MCF-7 Proliferation (IC ₅₀ , nM)
1	estradiol		1.00	inactive
	raloxifene		0.34	0.2
	tamoxifen		0.010	530
				
4a	HO	CO ₂ Me	0.07	50
4b	HO	CO ₂ Et	0.06	50
4c	HO	CO ₂ H	0.012	325
4d	HO	CONH ₂	0.039	200
4e	HO	CONHMe	0.016	40
4f	HO	CONMe ₂	0.040	20
4g	HO	COMe	0.075	32
4h	HO	PO(OEt) ₂	0.010	210
4i	HO	C≡CH	0.12	0.8
4j	HO	CH=CH ₂	0.10	7
4k	HO	C≡N	0.18	8
5a	MeO ₂ C	OH	<0.01	30
5b	<i>n</i> -BuO ₂ C	OH	<0.01	40
5c	<i>i</i> -BuO ₂ C	OH	<0.01	40
5d	HO ₂ C	OH	inactive	inactive
5e	H ₂ NOC	OH	<0.01	1000
5f	MeOC	OH	0.008	60
5g	(EtO) ₂ OP	OH	<0.01	200
5h	HC≡C	OH	0.029	20
6a	MeO ₂ C	CO ₂ Me	<0.01	1000
6b	H	CO ₂ Me	inactive	1000
6c	MeO ₂ C	H	<0.01	1000

References and Notes

1. (a) Riggs, B. C.; Melton, L. J. *New Engl. J. Med.* **1992**, *327*, 620; Dempster, D. W.; Lindsay, R. *Lancet* **1993**, *341*, 797; Lindsay, R. *Lancet* **1993**, *341*, 801; (b) Fink, G.; Sumner, B. E. H. *Nature (London)* **1996**, *383*, 306; (c) Pennisi, E. *Science* **1996**, *273*, 1171; (d) Love, R. R.; Mazess, R. B.; Barden, H. S.; Epstein, S.; Newcomb, P. A.; Jordan, V. C.; Carbone, P. P.; DeMets, D. L. *New Engl. J. Med.* **1992**, *326*, 852; (e) Polossek, T.; Ambros, R.; von Angerer, S.; Brandl, G.; Mannschreck, A.; von Angerer, E. *J. Med. Chem.* **1992**, *35*, 3537; (f) Anstead, G. M.; Wilson, S. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **1989**, *32*, 2163; (g) Lovely, C. J.; Gilbert, N. E.; Liberto, M. M.; Sharp, D. W.; Lin, Y. C.; Brueggemeier, R. W. *J. Med. Chem.* **1996**, *39*, 1917.
2. (a) 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.* **1994**, *93*, 63; (b) Grese, T. A.; Bryant, H. U.; Cole, H. W.; Kim, J. R.; Magee, D. E.; Rowley, E. R.; Sato, M. *J. Bone Miner. Res.* **1995**, *10*, S458 (suppl 1); (c) Termine, J. D. Presented at the American Society of Bone and Mineral Research, 16th Annual Meeting, Kansas City, MO, 1994, Minisymposia A: *Estrogens and Anti-estrogens*; (d) Draper, M. W.; Flowers, D. E.; Huster, W. J.; Neild, J. A. *Proceedings of the Fourth International Symposium on Osteoporosis*, 1993, 119; (e) Grese, T. A.; Dodge, J. D. *Ann. Reports Med. Chem.* **1996**, *31*, 181.
3. Dodge, J. A.; Lugar, C. W.; Cho, S.; Short, L. L.; Sato, M.; Yang, N. N.; Spangle, L. A.; Martin, M. J.; Phillips, D. L.; Glasebrook, A. L.; Osborne, J. J.; Bryant, H. U.; Frolik, C. A. *J. Steroid Biochem. Molec. Biol.*, in press.
4. Shakespeare, W. C.; Johnson, R. P. *J. Am. Chem. Soc.* **1990**, *112*, 8578.
5. Dolle, R. E.; Schmidt, S. J.; Kruse, L. I. *J. Chem. Soc., Chem. Commun.* **1987**, 904.
6. (a) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989; (b) Levi, E. M.; Mao, C. L.; Hauser, C. R. *Can. J. Chem.* **1969**, *47*, 3671.
7. Cabri, W.; De Bernardinis, S.; Francalanci, F.; Penco, S.; Santi, R. *J. Org. Chem.* **1990**, *55*, 350.
8. Thurieau, C.; Simonet, S.; Paladino, J.; Prost, J. F.; Verbeuren, T.; Fauchere, J. L. *J. Med. Chem.* **1994**, *37*, 625.
9. Cabri, W.; Candiani, I.; Bedeschi, A.; Santi, R. *J. Org. Chem.* **1990**, *55*, 3654.
10. Chambers, M. R. I.; Widdowson, D. A. *J. Chem. Soc., Perkin Trans. I* **1989**, 1365.
11. Cabri, W.; Candiani, I.; Bedeschi, A.; Santi, R. *J. Org. Chem.* **1992**, *57*, 3558.
12. Taylor, E. C.; Wong, G. S. K. *J. Org. Chem.* **1989**, *54*, 3618.
13. Glasebrook, A. L.; Phillips, D. L.; Sluka, J. P. *J. Bone Miner. Res.* **1993**, *8*, S268 (suppl 1); Thompson, E. W.; Reich, R.; Shima, T. B.; Albini, A.; Graf, J.; Martin, G. R.; Dickson, R. B.; Lippman, M. E. *Cancer Res.* **1988**, *48*, 6764.
14. Lindstrom, T. D.; Whitaker, N. G.; Whitaker, G. W. *Xenobiotica* **1984**, *14*, 841.
15. Full experimental details and results from additional biological assays will be reported (Grese, T. A. in press).