A CHEMICAL PROBE FOR THE ESTROGEN RECEPTOR: SYNTHESIS OF THE ^{3}H-ISOTOPOMER OF RALOXIFENE

Jeffrey A. Dodge,* Mark G. Stocksdale, C. David Jones

Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, IN 46285

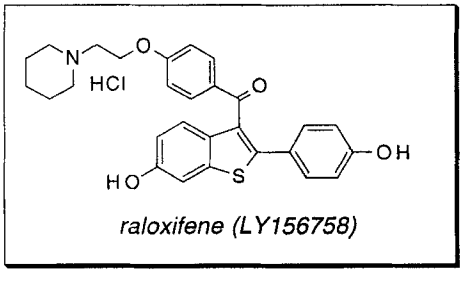
Key Words: anti-estrogen, raloxifene, osteoporosis, tritium, estrogen receptor

Summary

Radiolabelled raloxifene (LY156758) has been prepared by tritium gas hydrogenolysis of a 3-aroyl-*bis*-brominated precursor. The requisite halogenated intermediate was accessed by regioselective aroylation of benzothiophene **6** with the acid chloride of 3,5-dibromo-4-[2-(1-piperdinyl)ethoxy]benzoic acid (**5**). Selective deprotection of the aryl methyl ethers in the presence of the ethoxy side-chain followed by palladium catalyzed halogen-tritium exchange provided the target compound with a specific activity of 30.1 Ci/mmol.

Introduction

The estrogen receptor (ER) is a nuclear regulatory protein which mediates the actions of estrogen in target cells. Recognition of estrogens by the steroid binding domain of the ER results in activation of the complex and, ultimately, modulatory effects on gene transcription (1). Anti-estrogens (AEs) represent a unique class of compounds which act to agonize and/or antagonize these effects by competing with estrogen for ER binding thereby altering the normal course of gene activation (1b, 2). Remarkably, many AEs illicit their biological responses in a tissue selective manner, i.e., these compounds often block the endogenous effects of estrogen in reproductive tissues



raloxifene (LY156758)

CCC 0362–4803/95/010043–07
©1995 by John Wiley & Sons, Ltd.

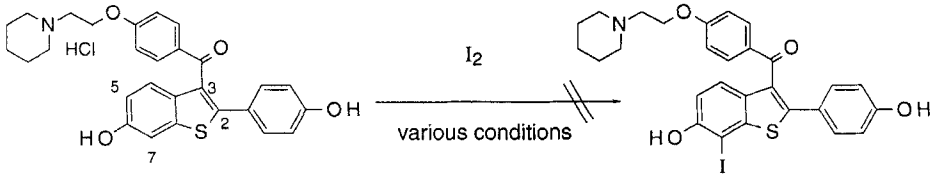
Received 15 June, 1994
Revised 15 August, 1994

but mimic estrogen in other pharmacological parameters. This dichotomy is exemplified by raloxifene (LY156758), a compound with estrogen antagonist effects in the breast and uterus (3) but stark agonist characteristics in bone metabolism and lipid lowering (4). This unique pharmacology has made raloxifene particularly attractive as a therapeutic agent for breast cancer (5) and, more recently, as a potential therapy for post-menopausal osteoporosis (4, 6). While this agonist/antagonist behavior is presumably mediated via the estrogen receptor, the molecular origin(s) of raloxifene's tissue selective behavior remains unclear. To address this issue, we have prepared ^{3}H-raloxifene to quantitatively examine the interaction of raloxifene with the estrogen receptor, as well as study the metabolism and disposition of this important drug in laboratory animals.

Results and Discussion

Despite wide-spread interest in the unique tissue selective properties of raloxifene (evidenced by over 200 literature citations to this compound), its synthesis in radiolabelled form has not been previously reported. We were therefore interested in developing a synthetic route amenable to preparing practical quantities of tritiated material. Initially, we envisioned two distinct strategies towards this end, both of which were designed to incorporate tritium late in the reaction sequence via a palladium-catalyzed tritium gas hydrogenolysis of the appropriate halogenated precursor. In one approach, the requisite halogenated intermediate would be prepared directly from raloxifene itself. In a second more convergent strategy, the desired halogen functionality is introduced at a much earlier stage in the synthesis.

Initial studies focused on incorporating the requisite halogen into the benzothiophene nucleus via direct electrophilic aromatic iodination of raloxifene. It seemed likely the substitution would occur on the electron-rich benzo[b]thiophene ring rather than the 2-aryl substituent or the electron deficient 3-aroyl group. However, it was unclear as to whether the C-5 or C-7 positions would be the favored site of attack since each position is activated by both the phenol and sulfur moieties. Calculations in a related system have shown that C-7 has a significantly higher HOMO coefficient than C-5 and thus would be the predicted site for electrophilic attack (7). Moreover, Katzenellenbogen has observed exclusive electrophilic iodination at C-7 in a similar benzothiophene

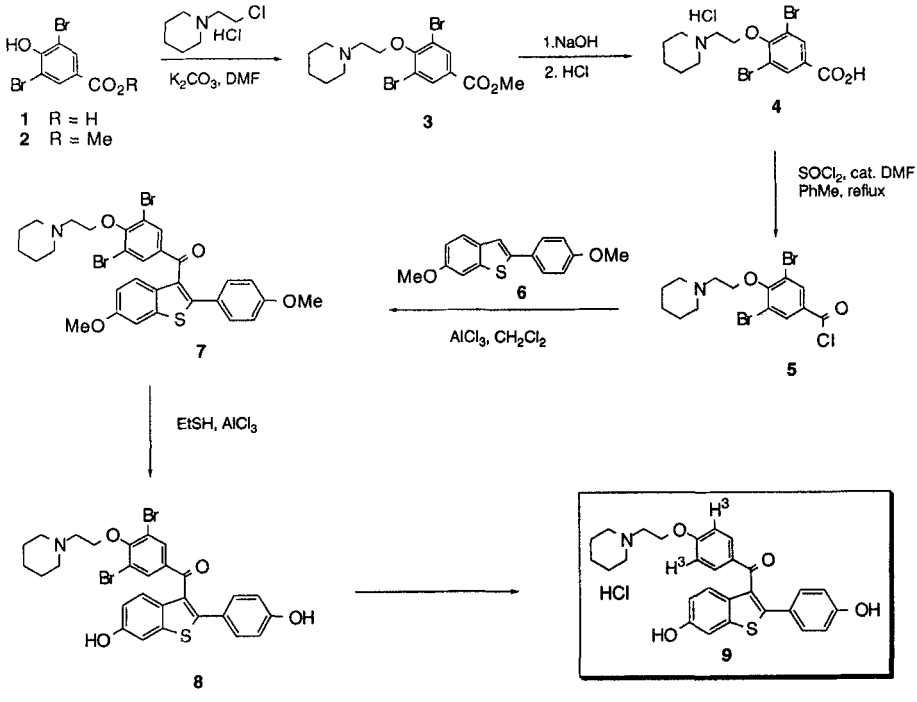


presumably due to anchimeric assistance by the adjacent sulfur atom. Despite these precedents, when we subjected raloxifene to iodination under a variety of experimental conditions none of the desired 7-iodo-raloxifene was obtained and starting material was invariably returned unchanged. This was largely attributed to the marked insolubility of the substrate in the solvents required for the iodination protocol.

As direct substitution on the benzo[b]thiophene of raloxifene ring proved unfruitful, an alternative synthetic strategy was examined in which the pre-requisite halide functionality is incorporated in the 3-aroyl moiety. Along these lines, benzo[b]thiophenes are well-documented to undergo aroylation at the 3-position under Friedal-Crafts conditions (3). Thus, reaction with an appropriately functionalized acid chloride would provide access to the desired 3-aroyl-2-aryl-benzo[b]thiophenes. Commercially available 3,5-dibromo-4-hydroxybenzoic acid (**1**) was selected as an ideal starting material. The two bromo-substituents provide the requisite functionality for halogen-tritium exchange while the phenol moiety allows for synthetic access to the piperdinoethyl pharmacophore.

As shown in Scheme I, 3,5-dibromo-4-hydroxybenzoic acid (**1**) is initially converted to the corresponding methyl ester (**2**) by treatment with methanolic hydrochloric acid. Subsequent reaction

Scheme I



of the phenol with N-(2-chloroethyl)piperidine under basic conditions gives **3** which upon saponification and acid treatment provides amino acid **4**. Friedel-Crafts reaction of the corresponding acid chloride (**5**) with benzo[b]thiophene **6** (3) and an excess of Lewis acid (aluminum chloride) proceeds with complete regiocontrol at the desired 3-position to provide **7** in highly convergent fashion. Selective deprotection of the aryl methyl ethers in preference to the piperidinoethyl side-chain is then accomplished employing Jones' ethane thiol/$AlCl_3$ protocol (3). This material (**8**) was then subjected to tritium gas hydrogenolysis and subsequently acidified (HCl) to provide *bis*(^{3}H)-raloxifene (**9**) with specific activity of 30.1 Ci/mmol and radiopurity of >99 % (HPLC).

In summary, radiolabelled raloxifene has been prepared in a highly convergent manner. Key transformations include regioselective aroylation of the benzo[b]thiophene nucleus followed by selective removal of the methyl ether groups in the presence of the ethoxy side chain. The availability of **9** as a chemical probe for the estrogen receptor has provided a valuable tool for examining the binding characteristic of AEs at the molecular level. For example, estrogen receptor binding studies with a series of of steroidal and non-steroidal AEs (raloxifene, tamoxifen, and ICI 164,384) in MCF-7 lysates has revealed a distinct binding site for raloxifene. This high affinity site is distinct for raloxifene and different from the well-documented tamoxifen, or anti-estrogen, binding site (8).

Experimental

General. All reagents were obtained from commercial sources and used without further purification unless otherwise indicated. ^{1}H–NMR and ^{13}C–NMR were measured as indicated at 300 and 75 respectively on a General Electric QE 300. ^{1}H–NMR chemical shifts are reported as δ values in ppm relative to the NMR solvent employed. ^{1}H–NMR coupling constants are reported in Hertz (Hz) and refer to apparent multiplicities. Multiplicity is indicated as follows: s (singlet); d (doublet), t (triplet), q (quartet); m (multiplet); comp (complex), br (broad), and app (apparent). Column chromatography was performed according to the method of Still (9) with EM Science silica gel (230-400 mesh ASTM). Radial chromatography was performed on a Chromatotron using 1, 2, or 4 mm silica gel coated plates. All air and/or moisture sensitive reactions were run under an argon or nitrogen atmosphere in rigorously dried glassware and anhydrous solvents. In all cases, concentrations were performed under reduced pressure with a rotary evaporator.

Methyl-3,5-dibromo-4-hydroxybenzoate (2). To MeOH (1 L) stirring at –30 °C was added acetyl chloride (50 mL, 0.70 mol) in a dropwise fashion. After the addition was complete, 3,5-dibromo-4-hydroxybenzoic acid (25 g, 84.5 mmol) was added all at once as a solid and the resulting solution allowed to warm to room temperature. After 18 h, the reaction was concentrated to give 25 g (95 %) of **2** as a white solid which was used without further purification: ^{1}H–NMR (300 MHz, DMSO-d_6) δ 7.98 (s, 2H), 3.79 (s, 3H); ^{13}C–NMR (75.5 MHz, DMSO–d_6) δ 164.1, 156.7, 133.1, 121.2, 111.9, 52.2; IR (KBr) 3350, 3083, 2950, 1707, 1309, 1262, 1128, 761; MS (FD) 308 (2 x Br^{79}), 310 (Br^{79}, Br^{81}), 312 (2 x Br^{81}); Analysis calcd for $C_8H_6O_3Br_2$: C, 31.00; H, 1.95; found: C, 30.85; H, 2.02.

3,5-Dibromo-4-[2-(1-piperidinyl)ethoxy]benzoic acid Hydrochloride (4). To a solution of **2** (10 g, 32 mmol) stirring in DMF was added potassium bicarbonate (22.2 g, 161 mmol). The mixture was heated to 100 °C and N–(2–chloroethyl)piperdine hydrochloride (7.42, 40.0 mmol) was added in three equal portions over 0.5 h. After 1 h, additional potassium bicarbonate (11.1 g, 80 mmol) was added and the mixture stirred vigorously at 100 °C. After 1h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated and the resulting light brown oil (**3**) used without further purification. To a solution of the methyl ester (11.8 g, 29.8 mmol) stirring in MeOH (120 mL) at 0 °C was added NaOH (35 mL of a 5N aqueous solution). The solution was then allowed to warm to room temperature and stirred overnight. The reaction mixture was then concentrated and the resulting residue dissolved in water (130 mL) then cooled to 0 °C. To this solution was slowly added HCl (40 mL of a 5 N aq. solution). The resulting white precipitate was collected by vacuum filtration to give11.0 g of **4** (83 %) as an off-white solid: ^{1}H–NMR (300 MHz, DMSO–d_6) δ 8.15 (s, 2H), 4.39 (t, J = 5.2 Hz, 2H), 3.51 (t, J = 4.7 Hz, 2H), 3.10-3.40 (m, 4H), 1.75-1.81 (m, 4H), 1.51-1.57 (m, 2H); IR (KBr) 3417, 2951, 2864, 2642, 2552, 1717, 1370, 1251, 1200, 737, 650 cm^{-1}; MS (FD) 406 (M^+ – HCl, Br^{79}), 408 (M^+ – HCl, Br^{79}, Br^{81}), 410 M^+ – HCl, Br^{81}); analysis calc. for $C_{14}H_{18}NO_3ClBr_2$: C, 37.91; H, 4.09; N, 3.16; found: C, 38.02; H, 3.99; N, 2.91.

[6-Methoxy-2-(4-methoxyphenyl)benzo[b]thien-3-yl][3,5-dibromo-4-[2-(1-piperidinyl)ethoxy]phenyl] methanone (7). To a suspension of **4** (0.44 g, 1.00 mmol) stirring in anhydrous toluene (10 mL) was added DMF (one drop) followed by thionyl chloride (0.73 mL, 10 mmol). The reaction mixture was brought to reflux for 0.5 h, cooled to room temperature, and concentrated to give a pale white solid (**5**) which was used with no further purification. [^{1}H–NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 2H), 4.63-4.66 (m, 2H), 3.71-3.75 (m, 2H), 3.51-3.56 (m, 2H), 2.87-2.95 (m, 2H), 2.23-2.35 (m, 2H), 1.55-1.89 (m, 4H)]. To a solution of the acid chloride (**5**) (0.46 g,

1.00 mmol) stirring in anhydrous CH_2Cl_2 (10 mL) was added **6** (0.18 g, 0.67 mmol). Aluminum chloride (0.80 g, 6.03 mmol) was then added in three equal portions over a 10 min period. After 18 hours at room temperature, the reaction mixture was poured into ice water (20 mL) and extracted with warm CH_2Cl_2 (3 x 10 mL). The combine organic extracts were washed with saturated aq. sodium bicarbonate, dried ($MgSO_4$), concentrated, and filtered. The crude material product was purified by radial chromatography (4 mm, 5% MeOH/ethyl acetate) to give 0.36 g (81%) of **7** as a yellow foam: ^{1}H–NMR (300 MHz, DMSO–d_6) δ 7.68-7.73 (m, 4H), 7.24 (d, 2H, J = 8.6 Hz), 7.07 (dd, 1H, J = 8.8 Hz, J = 2 Hz), 6.83 (d, 2H, J = 8.6 Hz), 3.97 (t, 2H, J = 6 Hz), 3.84 (s, 3H), 3.69 (s, 3H), 2.66 (t, 2H, 6 Hz), 2.32-2.40 (m, 4H), 1.30-1.42 (m, 6H); IR ($CHCl_3$) 2941, 1647, 1607, 1477, 1256, 1051, 831; MS (FD) 658 (M^+, 2 x Br^{79}), 660 (M^+, Br^{79}, Br^{81}), 662 (M^+, 2 x Br^{81}). HRMS (CI) calcd for $C_{30}H_{30}NO_4SBr_2$ 658.0262, found 658.0220.

[6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl][3,5-dibromo-4-[2-(1-piperidinyl)ethoxy]phenyl]methanone (8). To a solution of **7** (0.10 g, 0.15 mmol) dissolved in anyhdrous CH_2Cl_2 (5 mL) was added ethanethiol (0.06 ml, 0.80 mmol), followed by aluminum chloride (0.12 g, 0.91 mmol). The reaction was stirred vigorously for 30 minutes and then quenched with brine and saturated aq. sodium bicarbonate. The remaining residue was dissolved in a minimum amount of MeOH and the combined mixture extracted with ethyl acetate (3 x 25 mL). The combined organic extracts were washed with saturated aq. potassium sodium tartrate (3 x 15 mL) and brine (2 x 10 mL). After drying ($MgSO_4$), the solution was filtered, concentrated, and subsequently purified by radial chromatography (2 mm, 13:4:2:1 ethyl acetate/hexanes/ MeOH/triethylamine) to give 0.07 g (74%) of **8** as a yellow foam: ^{1}H–NMR (300 MHz, MeOHd4) δ 7.81 (d, 1H, J = 8.8 Hz), 7.70 (s, 2H), 7.27 (d, 1H, J = 2.3 Hz), 7.09 (d, 2H, J = 8.5 Hz), 6.95 (dd, 1H, J = 8.8 Hz), 6.61 (d, 2H, J = 8.5 Hz), 4.07 (t, 2H, J = 5.9 Hz), 2.88 (t, 2H, J = 5.7 Hz), 2.61-2.65 (m, 4H), 1.60-1.66 (m, 4H), 1.47-1.52 (m, 2H); MS (FD) 631 (M^+, 2 x Br^{79}), 633 (M^+, Br^{79}, Br^{81}), 635 (M^+, 2 x Br^{81}); analysis calc. for $C_{28}H_{25}NO_4SBr_2$: C, 53.27; H, 3.99; N, 2.22; found: C, 53.33; H, 4.24; N, 2.50.

(6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl][3,5-*bis*(^{3}H)-4-[2-(1-piperidinyl)ethoxy]phenyl]methanone Hydrochloride (9), [*Bis*(^{3}H)-raloxifene HCl]. A solution of **8** (20 mg, 0.03 mmol) in MeOH (2 mL) containing triethylamine (0.05 mL) was reacted at room temperature with 5% Pd/C (20 mg) and tritium gas. After 3.0 h, the catalyst was filtered off and labile tritium was removed by several evaporations with methanol. The resulting crude product was

diluted in methanol and purified by HPLC on a Zorbax ODS column eluted with 10 mM triethylammonium acetate/acetonitrile (65/35). The combined fractions were concentrated, diluted with ethanol, and HCl added to give the title compound (**9**) with a radiochemical purity of 99 % and specific activity of 30.1 Ci/mmol. The ^{1}H-NMR was identical to an authentic sample of raloxifene: (300 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.87 (s, 1H), 9.87 (s, 1H), 7.69 (d, J = 9.0 Hz, 2H), 7.37 (d, J = 2 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 7.17 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 9 Hz, 2H), 6.87 (dd, J = 9.0, 2.0 Hz, 1H), 6.70 (d, J = 9.0 Hz, 2H), 4.44 (m, 2H), 3.42 (m, 4H), 2.96 (m, 2H), 1.77 (m, 4H), 1.70, 1.36 (m, 2H).

Acknowledgement

We are grateful to Dr.'s Chuck Frolik and Bill Wheeler for purity determination and stability studies. Also, the Physical Chemical Research Department at Lilly is gratefully acknowledged.

References and Notes

1. For reviews see (a) Evans, R. M.– Science 240: 889 (1988). (b) Katzenellenbogen, B. S.; Miller, M. A.; Mullick, A.; Sheen, Y. Y. – Breast Cancer Res. Treat. 5: 231 (1985).

2. (a) Kumar, V.; Green, S.; Stack, G.; Berry, M; Jun, J.-R.; Chambon, P. – Cell 51:, 941 (1987). (b) Beato, M. – Cell 56: 335 (1989).

3. Jones, C. D.; Jevnikar, M. G.; Pike, A. J.; Peters, M. K.; Black, L. J.; Thompson, A. R.; Falcone, J. F.; Clemens, J. A. – J. Med. Chem. 27: 2057 (1984).

4. (a) Black, L. J.; Sato, M.; Rowley, R. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffman, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, C. A.; Bryant, H. U. – J. Clin. Invest. 93: 63 (1994). (b) Evans, G.; Bryant, H. U.; Magee, D.; Sato, M.; Turner, R. T. – Endocrinology 134: 2283 (1994).

5. Black, L. J.; Jones, C. D.; Falcone, J. F. – Life Sci. 32,: 1031 (1983).

6. For results of the Phase II clinical trial see: Draper, M. W.; Flowers, D. E.; Huster, W. J.; Neild, J. A. – Proceedings Fourth International Symposium on Osteoporosis and Consensus Development Conference, 1993, Christiansen, C. and Riis, B. eds, pp 119-123.

7. Pinney, K. G.; Katzenellenbogen, J. A. – J. Org. Chem. 56: 3125 (1991).

8. Glasebrook, A. L.; Phillips, D. L.; Sluka, J. P. – J. Bone Miner. Res. 8 (Suppl 1): S268 (1993).

9. Still, W. C.; Kahn, M.; Mitra, A. – J. Org. Chem. 43, 2923 (1978).

10. The tritiation and purification of **9** was conducted at New England Nuclear, Boston, Mass.